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PROCEEDINGS

SPRI 2004

CONFERENCE ON SUGAR PROCESSING RESEARCH



New Developments

APRIL 4-7, 2004
ATLANTA, GEORGIA

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INDEXING



**PROCEEDINGS OF THE
2004 SUGAR PROCESSING
RESEARCH CONFERENCE**

New Developments

**APRIL 4-7, 2004
ATLANTA, GEORGIA**

**Sponsored by
Sugar Processing Research Institute, Inc.
New Orleans, Louisiana**

September 2004

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PREFACE

The 2004 Sugar Processing Research Conference is one in a series of Conferences held in alternate years to provide a forum for the exchange of information among technical leaders of the sugar industry and to report on new and noteworthy developments. The Conference was sponsored by Sugar Processing Research Institute, Inc. (SPRI).

The program for this Conference was arranged by Mary An Godshall. The Conference Coordinators were Shirley Saucier and Xavier Miranda. These Proceedings were edited by Mary An Godshall.

Sugar Processing Research Institute, Inc., acknowledges the contribution in kind to the support of this conference by the Southern Regional Research Center, Agriculture Research Service, United States Department of Agriculture. We also gratefully acknowledge the support of the Coca Cola Company, who provided a very informative tour of their technical center and lunch for the delegates, along with much help during the planning phases of the Conference.

The series, Proceedings of the Sugar Processing Research Conference, of which this is the twelfth issue, continues the Proceedings of the Technical Session on Cane Sugar Refining Research, which was published every other year from 1964 to 1980. For individual copies of this volume, as well as back issues of the former series, as long as the supply lasts, write to Sugar Processing Research Institute, Inc., 1100 Robert E. Lee Blvd., New Orleans, Louisiana 70124. Before 1986, Proceedings were published by the Agriculture Research Service, U.S. Department of Agriculture. Since 1988, Proceedings have been published by Sugar Processing Research Institute, Inc.

Mary An Godshall
Managing Director
Sugar Processing Research Institute, Inc.

Robert O. Hatch, Chairman
Sugar Processing Research Institute, Inc.

SUGAR PROCESSING RESEARCH INSTITUTE, INC.

Sugar Processing Research Institute, Inc., is an independent, non-profit research institute supported by the international sugarcane and sugarbeet production and refining industries and their supplier and user companies. The Institute is housed at the Southern Regional Research Center of the United States Department of Agriculture, Agriculture Research Service, under a Memorandum of Understanding with USDA. The association with USDA offers many synergies for the benefit of the sugar industry. The SPRI organization is unique in that it undertakes both beet and cane sugar processing research.

The history of the institute began in 1939 with formation of the Bone Char Research Project at the National Bureau of Standards in Washington, D.C., under the direction of Dr. Victor Dietz, with a mandate to study the, then new, sugar decolorizing adsorbent, bone char. In 1963, it moved to New Orleans, Louisiana, and became the Cane Sugar Refining Research Project, with Dr. Frank Carpenter as its director. At that time, the focus was exclusively on cane sugar processing issues. In 1981, under the direction of Dr. Margaret Clarke, its scope was greatly expanded when it became Sugar Processing Research, Inc, and included beet sugar and raw cane sugar manufacturing in its research portfolio. In 1991, it was renamed Sugar Processing Research Institute, Inc. Mary An Godshall became the Managing Director of the Institute in 2000. This Conference commemorated 65 years of continuous research for the sugar industry by SPRI.

2004

*These Proceedings published by
Sugar Processing Research Institute, Inc.
1100 Robert E. Lee Blvd.
New Orleans, Louisiana 70124*

This volume is
dedicated to the memory of
Earl J. Roberts
1913 - 2004

FORWARD

The theme of the 2004 Conference was “**New Developments.**” A wide range of topics is covered in the forty papers and poster presentations within these pages, reflecting the range of developments in the sugar industry over the last few years.

These Proceedings are dedicated to the memory of Earl J. Roberts, who worked with SPRI for many years and who was responsible for many of the new developments from SPRI.

The winner of the SPRI Science and Technology Award, Dr. Mohamed Mathlouthi, provides an overview of sucrose-water interactions, illuminating not only the chemical/theoretical underpinnings of the topic but also showing the practical implications for sugar drying and conditioning.

The Conference featured topics in white sugar quality, new products and new developments. Topics in sugar quality include a discussion on the effect of dextran in white sugar, new sucrose standards for beverage manufacturers, the composition of Indian plantation sugar, understanding sucrose - water activity-moisture relationships and the measurement and control of off-odors in beet sugar.

Among new products and new developments are included a paper on ethanol production by the ZeaChem process, an element of the sugarcane biorefinery concept being developed in Australia. Natural biocides and a natural flocculent for potential organic sugar production were introduced, as well as a new technology for regenerating activated carbon. Novel applications of bagasse fly ash for environmental remediation are discussed. Beet polysaccharide structure and new products are mentioned in two papers. New ideas on application of ion exchange softening to cane juice and on optimizing beet factories are also presented.

Analytical advances are discussed in papers on NIR systems, real-time instrumental measurement of crystalline sugar color, a rapid starch test for use in cane mills and refineries, and sensitive sucrose loss measurements across clarification and evaporators as well as in sugar mills in Brazil. A simple, standardized method to determine the activity of dextranase enzyme in the mill is presented. The effect of oxidizing agents on high molecular weight cane and beet colorants is discussed.

The Conference consisted of a mix of oral and poster presentations. Poster authors were invited to submit a full paper on their poster presentations for publication in these Proceedings.

We hope that the reader will find many interesting ideas in these pages.

Mary An Godshall
September 2004

TABLE OF CONTENTS

In Memoriam, Earl J. Roberts	1
SPRI Science and Technology Award Winner	3
Previous Winners of SPRI Awards	4
Chairman's Welcome and Introductory Remarks	5
Water-Sucrose Interactions, Quality of Crystals and the Storage Stability of Sucrose	7
Presentation by Winner of the SPRI Science and Technology Award	
Mohamed Mathlouthi	
The Sugar Industry in Between Tradition and the Future	19
Giorgio Mantovani	
Ethanol Production by the ZeaChem Process: An Element of a Sugarcane Biorefinery	27
Les A. Edye, B.P. Lavarack, P.A. Hobson, J.A. Blinco, J.J. Hodgson, W.O.S. Doherty and G.E. Bullock	
A New Technology for Regenerating Sugar Decolorizing Activated Carbon	37
Hugh McLaughlin	
Sustainable Technologies and Valuable New Polysaccharide-Based Products from Sugar Beet Pulp	80
Brett J. Savary, Kevin B. Hicks, Marshall L. Fishman, Arlen T. Hotchkiss and LinShu Liu	
Composition and Structure of Cell Wall Polysaccharides From Sugar Beet Grown Under Mediterranean Climate and Relation with Beet Processing	87
Maria El Amrani, Khalid Fares, Qamar R'Zina, Marie-Jeanne Crepeau and Jean-François Thibault	
The Effect of Ozone, Hydrogen Peroxide and Sulfite on Cane and Beet Macromolecules	111
Mary An Godshall and Marianne McKee	
Observations on Sucrose Water Activity - Moisture Relationships	128
Charles L. Schmalz and Michael L. Stroebel	
Dextran in Refined Sugar: Impact on Hard Candy Processing	138
Lynn Haynes, N. Zhou and W. Hopkins	
Dextran in White Sugar, A Comparison of Three Methods	147
Mary An Godshall, Marianne McKee, Sara J. Moore and Ron Triche	

Harmonization of Sucrose Standards for Beverage Manufacturers	161
Marie J. Tanner and Michael Finnerty	
Determination of Components of Insoluble Matter in White Sugar by Means of X-Ray Micro-Analyzer EDX	165
Maciej Wojtczak and Krzysztof Polański	
The Content of Saponins in White Sugar and Thick Juice Samples from Polish Sugar Factories	174
Bogusław Król, Joanna Milala, Maciej Wojtczak	
Comparison of Two Methods of Volatile Analysis for Determining the Causes of Off-odors in White Beet Sugars -- SPME and Headspace	183
Sara J. Moore, Mary An Godshall and Casey C. Grimm	
The Effect of Ozone and Air on Off-Odors in Beet Sugar	193
Emmanuel Duffaut, Mary An Godshall, and Casey C. Grimm	
Differentiating Cane White Sugar from Beet White Sugar Using Ion Chromatography Profiles	209
Gillian Eggleston, G. Pollach and R. Triche	
Composition of Indian Plantation White Sugar: Comparison to International Standards	215
Mary An Godshall	
Changes in Number 1 Liquor on Storage	221
Sara J. Moore, Mary An Godshall, and Marianne McKee	
Laboratory Clarification Tests Using Natural Flocculants: Potential Application for Organic Sugar Production	230
Jesus E. Larrahondo, C.F. Sánchez, C.O. Briceño, J.I. Victoria, and M.A. Godshall	
The Concept of Different Natural Antibacterials for the Sugar Industry	237
Guenter Pollach, Walter Hein and David Beddie	
Successful Application Points to Control Bacterial Infections Throughout Sugar Factories Using Beta Acids/Betastab®10A	260
David Beddie, N. Isles, T. Wirth, G. Pollach, and W. Hein	
The Impact of Using Rapid Action Biocides for Mill and Cane Sanitation on Quality of Sugar and Molasses	267
V.M. Kulkarni	
Investigation on the Efficiency of Carbonatation Sludge Addition Into Turkish Fuels for Desulfurization of Flue Gases	273
Jale Leblebici and Ferit Leblebici	

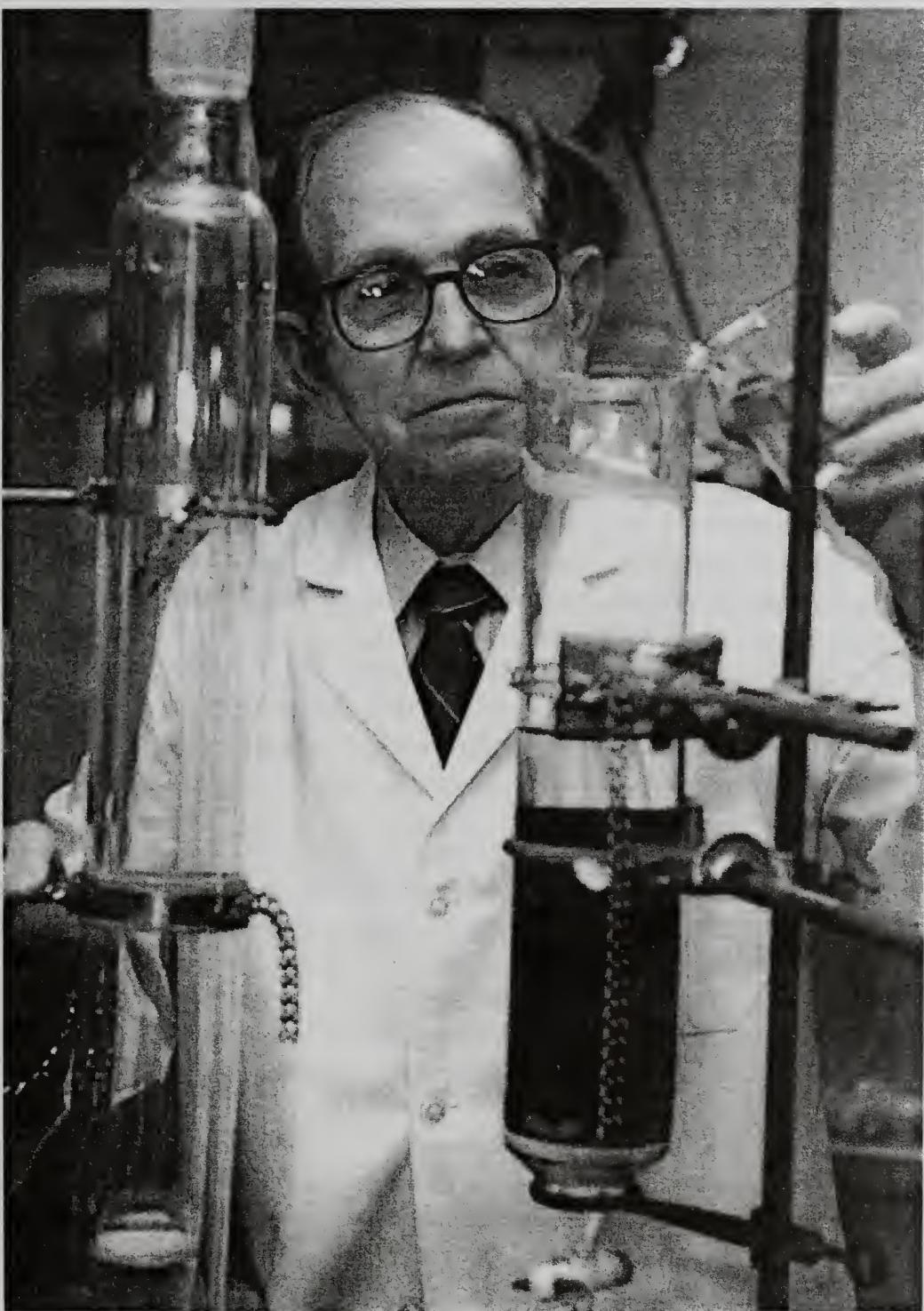
Real-time Solution Colour of Crystalline Sugar	283
Bjarne Chr. Nielsen	
Process and Energy Optimized Beet Sugar Factories	295
Reinhold Hempelmann, Andreas Lehnberger and Burkhard Bartels	
Possibilities for Ion Exchange Softening of Cane Clarified Juice	306
Vadim Kochergin, Jose F. Alvarez, William Jacob and Larry Velasquez	
Development of an On-line NIRS Procedure for the Analysis of Quality Parameters in Individual Consignments of Shredded Cane	315
Kevin J. Schäffler and R.C. Loubser	
The Application of Network NIR Calibration Equations at the Maryborough Sugar Factory .	328
S. Staunton, D. Mackintosh and G. Peatey	
Measuring Sugar Losses in Brazilian Mills	341
Henrique V. Amorim, L. F. Silva, C. Bernardino, A. Godoy, A.J. Oliveira, L.C. Basso and M.L. Lopes	
New Insights on Sucrose Losses Across Factory Evaporators and Juice and Syrup Clarifiers .	349
Gillian Eggleston, Michael Damms, Adrian Monge, and Trevor Endres	
Optimization of Sugarcane Factory Application of Commercial Dextranases in the U.S. .	371
Gillian Eggleston and Adrian Monge	
Determination of Extraneous Matter and Its Relationship to Different Harvesting Systems in the Colombian Sugar Agroindustry	395
Jesús E. Larrahondo, C.A. Viveros, C.O. Briceño and A. Patiño	
Molecular Probes for Assessing Boiling Difficulties	403
Emmanuel Duffaut and Mary An Godshall	
The Shape of Sucrose Molecules	417
Alfred D. French, Glenn P. Johnson, Anne-Marie Kelterer and Christopher J. Cramer	
A Rapid Starch Test for Use in Cane Mills	428
Mary An Godshall, Ron Triche and Sara J. Moore	
Collaborative Study on Starch in Raw Sugar Using the SPRI Rapid Starch Method	442
Mary An Godshall	
Application of a New GC-MS Method for Determining Ester Contents Following Alkaline or Enzymatic Hydrolysis of Sugar Beet Pulp and Pectin	449
Brett J. Savary and Alberto Nuñez	

Studies on Bagasse Fly Ash As an Adsorbent for Waste Materials	454
Marianne McKee and Mary An Godshall	
Critical Influence of pH on Polyol Production by <i>Hansenula anomala</i> in Sucrose Based Medium	461
S. V. Patil, R.V. Burase, V.K. Jayaraman and B.D Kulkarni	
Process Technological and Economical Integration of VTIR (Technological and Industrial Value of Beet): Harvesting, Washing and Processing	471
Manuel Ruiz Holst, Fernando Martin Dominguez and Marta Garcia de Quevedo	

In Memoriam

Earl J. Roberts

1913 - 2004



Earl J. Roberts

1913 - 2004

Earl J. Roberts was born in Magee, Mississippi, and grew up on the family farm. His father died when he was eleven years old, so, as a young boy, he had heavy responsibilities on the farm. In 1935, after graduating from high school, Earl entered Mississippi College at Clinton, Mississippi, where he played in the National Guard Band, worked on the campus, and in his third year, was appointed Laboratory Instructor in organic chemistry, to help pay his way. He graduated with distinction in 1939 with a major in chemistry. Employment as a Chemist was difficult to find at the time, so he took a job as a high school science teacher and later a job with the Mississippi Testing Laboratory testing concrete.

In 1941 he was appointed a graduate assistant in the Chemistry Department at Louisiana State University, from where he graduated in 1942 with an M.S. in organic chemistry. He then obtained employment at the U.S. Department of Agriculture, Southern Regional Research Center, in New Orleans, Louisiana, and begin a long and distinguished career. He first worked on preparing glue from cotton and peanut proteins, needed in the war effort. Several papers and patents resulted.

After two years, he was assigned to work on processing problems in the cane sugar industry. His first accomplishment was to develop a quantitative method for aconitic acid in sugar house products and then to help develop a commercial process for recovering aconitic acid from cane molasses. The Godchaux Sugar Company built an aconitic recovery plant at Raceland, Louisiana, based on this process, which operated for several years at considerable profit. Earl was awarded the USDA Superior Service Award in 1950 for this work. He continued in sugar research, on the isolation and identification of the non-sugars, including organic and amino acids, in sugarcane juice. He is responsible for developing the table on the Composition of Sugarcane that has appeared in the Cane Sugar Handbook since 1983.

The work on sugarcane processing was discontinued by USDA in 1965 and Earl went to work on the structure of cotton cellulose, leading to many publications and patents. When he retired from USDA in 1972, he went to work for the Cane Sugar Refining Research Project, predecessor of Sugar Processing Research Institute, until 2000. Among his many accomplishments at SPRI were elucidating the cause of acid beverage floc, determining the composition of indigenous sugarcane polysaccharide, developing DEAE bagasse, development of levan derivatives and development of the Roberts copper method for dextran determination, which is an official AOAC method of analysis. Earl won the SIT Crystal Award in 1990.

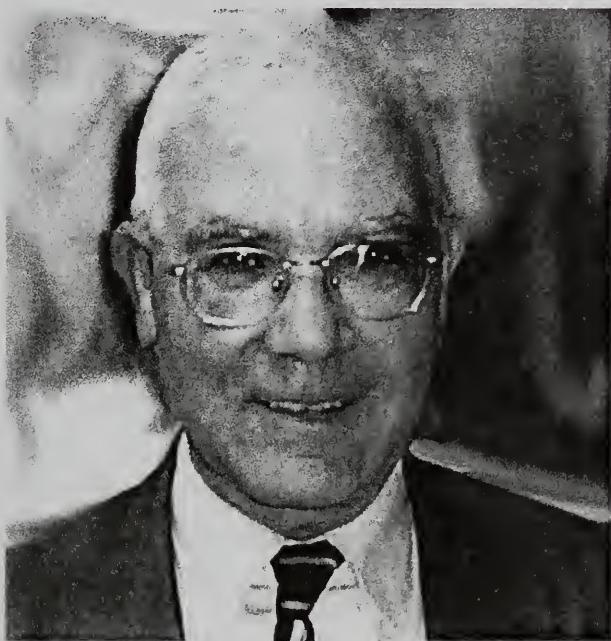
Earl brought a wealth of knowledge to his work, being able to synthesize all he had learned along the way. He also brought a quiet and gentle sense of humor and was a wonderful story teller.

Earl was especially proud of his invention of DEAE bagasse, with its superb decolorizing ability, and enjoyed demonstrating its capacity, as shown in the accompanying photograph.

2004 SPRI Science and Technology Award

MOHAMED MATHLOUTHI

Dr. Mohamed Mathlouthi, Professor of Industrial Physical Chemistry at the University of Reims in France, was born in Tunisia in 1940. In 1966, he completed his degree as an Engineer in Food Science and Technology at ENSIA in Massey, France. His lifelong association with sugar began when he worked as an engineer at the Beja Sugar Factory in Tunisia from 1966 to 1969.



Mohamed returned to France in 1969, as an Assistant Professor and later Associate Professor of Food Science and Technology at the University of Dijon, during which time he earned a doctorate in Engineering, with a specialty in the physical chemistry of foods. In 1980, Dr. Mathlouthi, in his continued quest for knowledge, obtained a second doctorate in Physical Chemistry from the University of Bourgogne in Dijon.

From 1986 to the present, Dr. Mathlouthi has been a distinguished Professor of Industrial Physical Chemistry at the University of Reims. The University is situated in the Champagne-Ardenne, the heart of the champagne district of France, and an ideal venue to pursue work on sugar.

His research interests are wide ranging, including water-carbohydrate interactions, sucrose crystallization and properties, solution properties and sweet taste chemoreception, and water activity and shelf-life of foods. In 1992, he organized the Andrew VanHook Symposium, an annual one-day event that brings together experts in a selected topic related to sugar. He has received many honors, including the bronze, silver and gold medals from the French Sugar Association in 1995, 1997 and 1999, respectively. He has authored or co-authored 130 technical papers, and is a member of numerous professional organizations.

Dr. Mathlouthi is married and has two children.

SPRI SCIENCE AND TECHNOLOGY AWARD

The SPRI Science and Technology Award is presented biennially to an outstanding scientist, whose research accomplishments are distinguished by their originality and their contribution to sugar processing and production. The Award is presented for the purpose of promoting science and technology in sugar processing and production.

Previous Winners of the SPRI Science Award

- 2002 **Benjamin L. Legendre**
The Quest for Quality in Louisiana Sugarcane and Sugar
- 2000 **Jean-François Thibault**
New Ways to Add Value to Sugar Beet Pulp
- 1998 **Markwart Kunz**
Sucrose - Raw Material for Chemistry and Biochemistry
- 1996 **Pascal A. Christodoulou**
Energy Economy Optimization in the Separation Processes of Sucrose-Water and Non-Sugars
- 1994 **Frieder Lichtenthaler**
Computer Simulation of Chemical and Biological Properties of Sucrose, the Cyclodextrins and Amylose
- 1992 **Riaz Khan**
Chemical and Enzymic Transformations of Sucrose
- 1990 **Giorgio Mantovani**
Growth and Morphology of the Sucrose Crystal
- 1988 **Leslie Hough**
Sucrose, Sweetness and Sucralose
- 1986 **Andrew Van Hook**
Events in Sugar Crystallization

Chairman's Welcome and Introductory Remarks

Dr. Charley Richard
Chairman of the Board
Sterling Sugar

Sugar Processing Research Institute (SPRI) has evolved from 1939 as the Bone Char Research organization with a very specific focus to the internationally recognized research facility we now know. Today, SPRI has a broad focus toward all aspects of sugar related research from both sugarcane and sugar beets. Therefore, membership of SPRI and the personnel who normally attend the biennial meetings represent a broad cross section of the international sweetener industry. This gathering of disciplines and backgrounds from around the world provides a unique opportunity to communicate on important issues that challenge all sugar industries today.

SPRI's evolution is a result of many researchers who have participated in its activities. Two of these people, Earl Roberts and Margaret Clarke were most instrumental in elevating the organization to its current prominent status. Numerous others have also assisted SPRI in solving the challenges faced by sugar industries around the world. As a result of these research efforts, a solid foundation was formed allowing SPRI to be recognized as a 'center of excellence'.

All sugar industries around the world are now attempting to improve their efficiency as they seek to be globally competitive. In many instances this effort has led to a reduction in what some corporate officers might consider to be unnecessary or less important expenditures. As a result, numerous sugar related research groups around the world have been eliminated or reduced in scope. Many of these were private research facilities while others were industry organizations. Since there are fewer sugar processing related facilities today, SPRI has an even larger role than ever. However, special challenges result because specific research initiatives are combined from both sugarcane and sugar beet industries representing the diversity from all of the sugar producing continents. While the reduction in research efforts is an alarming trend, SPRI's ability to cross geographical and crop boundaries in solving research issues becomes a very strong asset.

Collaborative research efforts can allow industries to streamline their programs and yet produce the results needed to allow them to maintain their level of efficiency. Conducting sugar processing related research on a collaborative basis offers some special challenges but is a solution to accommodate industry attempts to improve global competitiveness.

SPRI will continue its valuable research work. But, to improve its effort several things are needed. One is additional membership. There are numerous advantages to becoming a member of SPRI, not the least of which are the research results from specific objectives and communication with other sugar industries. The staff and officers of the organization are eager to discuss all of the benefits of membership. New members and their accompanying dues will allow for a larger research staff to solve more challenges. And, additional research will provide more technology that will yield more discussion at each of its meetings.

While SPRI has traditionally worked and will continue to work on sugar quality issues, there are many new challenges we now discuss. One of these involves biotech-produced sugar or sugar resulting from genetically engineered sugarcane or sugar beets. This is an important issue that will affect sugar industries in the very near future as sugarcane and sugar beet agricultural research moves to production of biotech derived sugarcane. A second topic involves alternate products that all industries attempt to examine as we seek additional revenues from the sugarcane or sugar beet crop. Food safety is an ever-increasing concern and SPRI's research program will undoubtedly work toward these issues as well.

Your goal as a past and future participant in SPRI's meetings is to encourage your own company to consider membership if it isn't already so. With a strong membership base, the organization can do more to help us all be competitive in the global community we are now in.

Having served as Chairman for the past two years has given me great insight as to the needs of many throughout the international sugar community. I see SPRI as a research and technology transfer organization that does "produce results". It can improve and will do so with the help of each of you. Membership in SPRI just may be the key that will unlock the doors to improving sugar processing in your own industry.

On a personal note, the staff at SPRI is truly dedicated to solving the challenges of the international sugar community. They are an extremely professional staff and are eager to assist you. I would like to thank them for making the role of chairman so easy and for conducting their work in a way that makes us proud to be a member of Sugar Processing Research Institute.

SPRI Science Award Keynote Address

WATER-SUCROSE INTERACTIONS, QUALITY OF CRYSTALS AND THE STORAGE STABILITY OF WHITE SUGAR

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ABSTRACT

Caking of white sugar can be considered as a spontaneous agglomeration phenomenon which occurs under certain conditions of quality of crystals (especially large C.V., broken crystals, presence of amorphous and fine particles) and characteristics of surrounding air (temperature, relative humidity).

To account for the interactions between water and sucrose in the thin layer of syrup surrounding the sugar crystal, water vapour sorption isotherms are obtained at different temperatures and the water content at the surface and inside the crystals determined using the Karl Fischer titration method.

To characterize the quality of crystals and the defects engendered during the manipulation of dry sugar in dryers, coolers, elevators and sieves, image analysis was used applying LUCIA software to the images of crystals obtained with Nikon binocular and camera.

A relation exists between the quality of sugar crystals and their flowability in silos determined with a Jenike shear cell. To thoroughly understand the mechanism of caking, there is a need of approaching both the macroscopic visual image of the crystals and the microscopic molecular interactions between water and sucrose.

INTRODUCTION

The stability of bulk white sugar is generally approached by sugar technologists as a matter of drying or removal of residual moisture by use of dehydrated air for the ventilation of the silo. In fact, reaching thermodynamic equilibrium between water and sucrose molecules in the film of syrup surrounding sugar crystals, which is the condition for stability, proves to be a more

complex phenomenon than the simple release of water in the atmosphere. The driving force for migration of moisture from sugar crystals should be defined in terms of water activity rather than the nominal concentration of water in the surrounding film of syrup. The hydration of sucrose and its clustering plays the major role in establishing the driving force of monomeric water release from the superficial film of syrup. What makes the problem of the behaviour of crystalline sugar during conditioning and storage difficult to deal with both from the practical and theoretical point of view is the frequent presence of an amorphous layer surrounding the thin film of saturated syrup. The simultaneous occurrence of heat and mass transfer at the surface of the crystal and the retarding effect of bound water migration by the crust of amorphous sugar are some of the causes of the caking phenomenon.

Another factor affecting the stability of crystalline sugar is the quality of crystals. This quality is commercially defined by the European points, which are based on the determination of aspect, colour in solution and ash. Other parameters are often used like the moisture content and the crystal size distribution. These quality criteria are highly dependent on the way crystallisation was conducted, the washing in the centrifugals was made as well as on the drying, cooling, and screening prior to conditioning and storage.

To control the storage stability of crystalline sugar, it is necessary that total water content and the repartition of moisture between inclusion and the surface of the crystal is determined. Likewise, water vapour adsorption isotherms and the image analysis of crystals help in the understanding of the origins of white sugar instability and especially to explain the causes of breakage of sugar crystals and dust formation. It is indeed evident that broken crystals and fine sugar particles are highly hygroscopic and actively contribute to the lumping of bulk white sugar.

WATER-SUCROSE INTERACTIONS

Molecular association in aqueous sucrose solutions

The water molecule, commonly viewed as a tetrahedron with a lone pair of electrons at each of two corners and positive hydrogen at the other two, has the capacity to form protonic bridges at each corner. This makes water a highly self-associating compound as well as solvating (hydrating) agent. Consequently, the sucrose molecule with its eight hydroxyl groups, three hydrophilic oxygen atoms and fourteen hydrogen atoms, can readily interact through hydrogen bonding with water as well as other molecules of sucrose. Therefore, at least three elementary types of molecular interactions take place in sucrose solutions: water-water, sucrose-water and sucrose-sucrose, all resulting in the formation of intermolecular hydrogen bonds. Collisions involving already formed associations and leading to even larger homo- or hetero-molecular aggregates are possible as well. Thus, in general, the system may contain the whole variety of chemical species, starting from free molecules of water and sucrose, through water or sucrose dimers, trimers, etc., then sucrose mono-, dihydrates, etc., and eventually ending with large polyhydrated clusters of sucrose.

The presence in aqueous solution of sucrose conformers due to the formation of intra-molecular hydrogen bonds deduced from experimental work by Mathlouthi (1981) and also from molecular simulation (Immel and Lichtenthaler, 1995) would make the overall picture even more complicated. The actual quantities of individual species in the solution depend on such conditions as temperature,

pressure and the nominal composition (sucrose-to-water ratio). For a detailed description of individual molecular interactions and structures occurring in aqueous solutions of sucrose the reader is referred to our recent review paper (Starzak, Peacock and Mathlouthi, 2000).

Water in the film of syrup surrounding the crystal and the process of drying sugar

Studies on the cluster composition of saturated water vapour, show that at normal P - T conditions encountered in sugar factory, the vapour is almost exclusively composed of water monomers (Visagin, 1983; Owicki et al., 1975). This implies that water clusters are practically nonvolatile and the process of water migration during sugar drying and conditioning is driven by the difference in water monomer concentration between the liquid and gas phase. In other terms, the driving force controlling drying should be expressed in terms of water activity rather than water content (Van Hook, 1987). On the other hand, migration of water from the superficial film to surrounding air is accompanied by simultaneous heat transfer, which makes the mass and heat transfers coupled during the drying of sugar. Moreover, depending on the conditions of crystallization and more importantly of the rate of drying, the morphology of the layer adjacent to the crystal may vary. The nature of water to remove by dehydration is generally found under 3 forms: *free water* corresponding to a thin film of unsaturated solution carried by each crystal when the sugar is discharged from the centrifugals; *bound water* corresponding to the layer of supersaturated solution trapped by the amorphous sugar crust during drying at high temperature; *inherent water* corresponding to the fraction of water embedded in the crystal lattice and resulting from the inclusion of droplets of mother liquor during the crystallisation step. During the storage of sugar, the bound water fraction represents an important factor of instability, especially if an amorphous crust is present at the surface of crystals.

QUALITY OF SUGAR CRYSTALS

The quality of crystals is appreciated visually or by the analysis of properties like solution coloration. From these observations, it is possible to determine the defects of sugar crystals, which can be classified as internal and external defects. Among internal defects, inclusions are the most important. External defects are those visually observed like twins, conglomerates, agglomerates and fragments of crystals obtained by the breakage of the most fragile needle-like single crystals.

Inclusions

The presence of impurities inside the crystal lattice has important effects on the sugar quality especially as concerns colour and ash content as well as the included fraction of moisture which may be released during storage and provokes caking under certain conditions. The study of inclusions is well documented (Vaccari and Mantovani, 1995). The origin of inclusions is mainly the capture of mother liquor droplets during fast growth of sugar crystals. In fact, the various faces of sucrose crystal have different rates of growth and the most rapid faces include mother liquor more rapidly than the others. It sometimes happens that the inclusion is not visible in the crystal. In such a case, heating at 105°C in the oven for more than one hundred hours can reveal the coloured drop included in the crystal lattice (Figure 1). The inclusion phenomenon can be limited by controlling the supersaturation of mother liquor to master growth kinetics.

External defects

The defects of crystals easily detected visually or under the microscope are morphological. External habits of sugar crystallized under various conditions are described in the Vavrinecz Atlas of Sugar Crystals (1965). Among the forms which are different from the simple 15 habits characterized by their Miller's indexes (Vavrinecz, 1965), there are different twins, conglomerates and elongated shapes.

Twins

In case of twins, 2 single crystals have a junction along a different plane depending on the type of twin (Vavrinecz, 1965)

- (a) Type 1 twin crystals have their left poles turned towards each other while the right poles point outwards
- (b) Type 2 twin crystals have their right poles grown together while the left poles are pointing outwards
- (c) Types 3 twin crystals have both single crystals placed behind each other and are grown together along the a face (Figure 2).

The junction zone between the single crystals in a twin is, from the mechanical point of view, a point of weakness where crystals can separate into fragments. Moreover, mother liquor retained between the crystals is not eliminated by washing in the centrifuge (Vaccari and Mantovani, 1995). Formation of twins depends on supersaturation. The higher the supersaturation, the more probable is twin formation.

Conglomerates

In such assembling of crystals, the junctions are random. Many crystals attached to each other grow together. This phenomenon happens when very high supersaturation is obtained locally in boiling pans. The consequences of this defect are the increase of colour in solution and included moisture as well as friability of crystals (Figure 3).

Agglomerates

After drying of sugar crystals, it happens that an amorphous layer of dry sugar is formed at the surface above a thin film of (super)saturated solution. This phenomenon together with the heterogeneity in moisture and the presence of fine particles lead to the agglomeration of two or more crystals. Here again the junction between particles includes moisture and can break during handling (Figure 4).

Other defects

Chalky white sugar crystals are observed when the amount of dust is high. This originates in case of abrasion by metal screens and scrolls or scratching in drum-dryers.

Irregular shapes, especially needles, are observed when sucrose is grown in the presence of specific impurities (raffinose, dextran). A large C.V. of sugar crystals can have as its origin the use of seeds with large size distribution, spontaneous nucleation and rapid changes in vacuum or temperature during boiling.

CONSEQUENCES OF CRYSTAL DEFECTS

The consequences of sugar crystal defects are numerous and economically detrimental. They may lead to the formation of fine particles by different breakage mechanisms (Verkoeijen et al., 2002). Depending on the force applied and its direction (i.e.; normal, tangential or any direction), the different breakage mechanisms observed are called attrition, abrasion, wear, fracture, fragmentation and chipping. Attrition (Figure 5a) and fragmentation (Figure 5b) are caused by normal forces, abrasion (Figure 5c) and chipping (Figure 5d) by tangential ones and wear and fracture by forces of any direction. The size of particle after attrition, abrasion or wear remains almost the same, but the shape becomes rounder. During chipping, small pieces of particles are broken and the particle becomes rougher. Fracture and fragmentation yield small fragments, thus reducing the average size of particles.

The formation of fragments of particles and the change in size and shape of crystals makes sugar crystals more reactive towards water vapour. This is directly linked to the aptitude of white sugar to cake. The most detrimental factor on the flowability of sugar was found to be a high amount of fine particles (above 10 %) (Rogé and Mathlouthi, 2003)

On the other hand, sucrose crystals are sometimes defined as hard or soft depending on their friability. In fact, hardness of sugar crystals is not well defined in the literature. A true hardness could be defined by an objective test such as the Brinnell index. Hardness according to Vickers for sucrose crystals is equal to 755 MPa (Bubnik et al., 1997). It seems that boiling at low temperature yields soft crystals as compared to normal or high temperatures. Depending on the size of crystals, the large crystals appear to be hard and sharp and the small crystals feel soft by comparison. All these defects result in an increased instability of bulk white sugar, especially as water vapour pressure is increased.

ANALYSIS OF PARAMETERS AFFECTING WHITE SUGAR STABILITY

Water Content

Water content in sugar crystals was analyzed using an adapted method of Karl Fischer titration. Total water was obtained after complete dissolution of the sugar sample in methanol/ formamide mixture (2/3 - 1/3 v/v) at 50°C (Rogé and Mathlouthi, 2000). After analysis of another sample of sugar and a very short agitation only allows release of surface water and its titration. This method permitted showing that about 80 % of moisture is located inside the crystal and only 20% at the surface. Moreover, water is not included in the crystal lattice as pure water but in mother liquor droplets observed by oven heating of crystals at 105°C (Figure 1). As already mentioned, included water increases the fragility of sugar crystals and yields fragments and dust.

Water Vapor Sorption Isotherms

The drying and handling of sugar crystals with defects such as conglomerates, twins and inclusions increases the probability of dust formation. Likewise screening of crystals especially when it is repeated to obtain a certain grain size (M.A.) may be at the origin of the abrasion of large crystals and the sticking of fine particles at the surface of these crystals (Figure 6). Although commercial requirements of a certain mean aperture (M.A.) and size distribution (C.V.) are met, the sugar behaves as very hygroscopic and is subject to caking. The role of fine particles in caking phenomenon has been published (Rogé and Mathlouthi, 2003). We report here a series of water vapor sorption curves (Figure 7) showing the role of fine particles added to standard sugar. Moisture content increases at the surface of sugar crystals which are covered with fine particles. These particles rapidly dissolve and recrystallize releasing free water. A chain reaction of dissolution and recrystallization is initiated, which ends with the lumping of the whole sample of sugar.

Image analysis

Image analysis can be a helpful and handy tool to determine and observe the breakage of sucrose crystals. Computer image analysis also enables evaluation of crystal size distribution and control of crystallization process in technical sugar solutions, which involves the evaluation of the produced crystals (Bubnik et al., 2001). The Laboratory Universal Computer Image Analysis - LUCIA G system, which was applied in this work, is a product of the LABORATORY IMAGING Co., Prague, Czech Republic. LUCIA G is the true color version and grabbing, processing and analysis of images are performed at RGB or HSI color space. LUCIA includes a powerful, full-featured macro language and together with a rich image analysis library provides an excellent developer's environment for image analysis.

Three different kinds of images can be measured: color, binary or so-called mask image. Color image is used for intensity measurement or tone intensity determination. Binary image is useful for shape determination or size measurement (area, length, elongation). Mask image helps to reduce some area of measurement.

A wide spectrum of different objectives, microscopes, cameras and lighting systems enables the measurement of particles with different size range from several centimeters to microns. The program involves a large amount of tools for adjustment and treating the image to obtain the form suitable for optimal computer analysis.

Other components of the system used for image analysis were: digital camera JVC TK-C1380 with a resolution of 470,000 pixels, card Mu Tech 400 that is able to transfer the data from the camera to the computer, system of lenses Navitar, stand Kaiser and fiber optic lamp Hund Wetzlar.

Parameters for size distribution measurement

To determine the crystal size distribution different parameters were used:

- *Area (A)* is the main measured size parameter. It is expressed as a number of pixels (for uncalibrated measurements) and gives the true size of the object (if the calibration is made).
- *Maximum and minimum feret diameter (MaxFer) and (MinFer)* are measured as a length of an object projection under the angle 0 – 180 ° (measured by 10°).
- *Equivalent diameter (EqD)* is determined by the circle diameter, which would be of the same area as a measured object: $EqD = (4 A / \pi)^{1/2}$
- *Elongation (E)* is determined as a ratio of maximum and minimum feret:

$$E = MaxFer / MinFer$$

Object evaluation and separation

The ability to separate and differentiate scanned objects is a basic parameter for object measurement, which shows also a quality of used image system. Above all, four main functions *erosion*, *dilatation*, *open* and *close* can be used for this purpose. After using *erosion* (inner layer of object is subtracted) and *dilatation* (outer layer of the object is added) the object size is reduced (*erosion*) and/or enlarged (*dilatation*) thus the results are affected. However using *open* (*erosion* followed by *dilatation*) and *close* (*dilatation* followed by *erosion*) the size of object is not affected. Other important functions are *Close Hole*, *Smooth Binary*, *Clean Binary*, *Contour* and others.

In many cases it is more useful to use a manual separation of objects. The LUCIA system enables shift between an original (unmodified) image and modified image during manual processing. Manual separation allows excluding irregular aggregates, which may be caused by imperfect sample preparation. In many difficult cases the operating personnel intervention is very meaningful and manual evaluation of the data depends strongly on experience and practice of the operating personnel.

Automatic measurement of scanned objects

The LUCIA system contains a program unit based on the programming language C++, which allows creating programs for evaluation and measurement of crystal size distribution and many others. All steps performed during picture evaluation are automatically saved in a subroutine and the whole process thus can be repeated if needed. Some steps can be changed or deleted. When the control of automatic evaluation is necessary, the program can be stopped and then modified or confirmed. The program has some additional functions like: addition of text labels for easier manipulation, limits and thresholds conditions (threshold setting, measurement of particles of a certain size, circularity utilization to exclude air bubbles, fibres from packages or admixtures), addition of different calculations for size, weight and area determination according to created relations, etc.

Result output

Output of results might be in the form of a table, histogram, or it can be transferred into a spreadsheet for further processing. The program enables a wide choice of evaluation parameters including statistical processing. All obtained images and tables can be saved and processed

again. Additional scale, description, text label or legend can be added into images. An up-grade LUCIA version saves all original images and supplemental information in two levels so that measured image can be displayed in two versions, the original one and the modified one as well. Study of the crystal breakage mechanism by image analysis

As was mentioned, the LUCIA system enables capture of color pictures of different objects and can be used to observe and compare different breakage mechanisms. Fig. 8 shows histograms (equivalent diameter distribution) of sucrose crystals before and after breakage in dependence on period of breaking. For comparison, pictures of broken crystals, from which the data for distribution measurement were taken, are displayed next to the corresponding histogram as well. It is clearly demonstrated that breakage yields a large C.V. and an increase in dust particles (< 150 μm).

CONCLUSION

Localization of moisture inside the crystal and the establishing of water vapour adsorption isotherms helps understanding of the role of crystal defects on caking. Storage stability of sugar depends on the quality of crystals visually manifested by defects (twins, conglomerates, inclusions) as well as on the presence of amorphous or fine particles around the crystals. Analysis of the causes of sugar instability reveals the role of surface water, the fine particles and the origin of dust particles as analysed by LUCIA image analysis. Image analysis of sugar crystals allows direct observation of the defects. It can help in determining the breakage mechanism by establishing crystal size distribution histograms.

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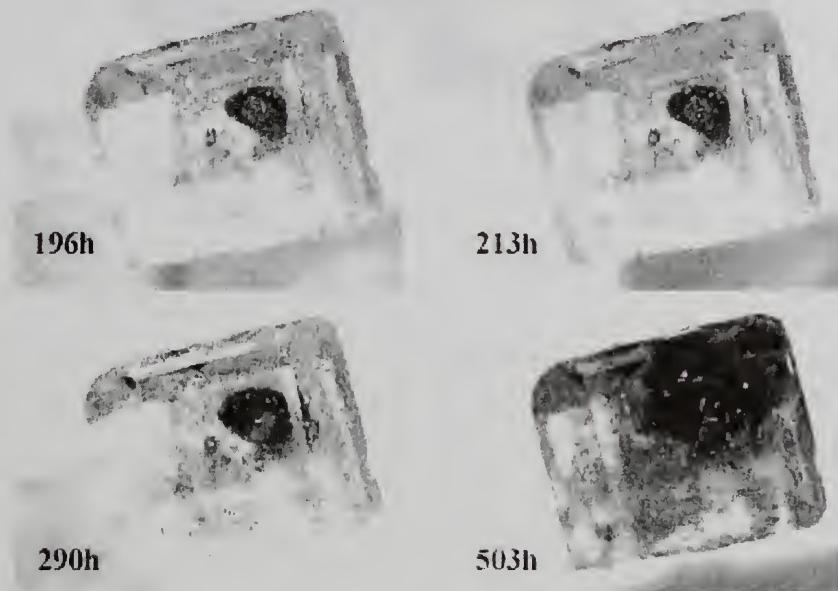


Figure 1 : Included water revealed by a thermal treatment at 105°C.



TWINS (Type 3)

Figure 2 : twin (Type 3) crystals are grown together along the \bar{a} face .

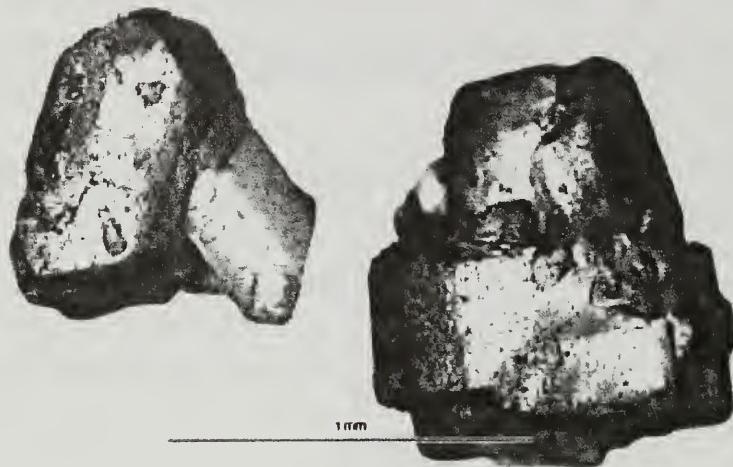


Figure 3 : Conglomerates : random junction



Figure 4 : Agglomerates

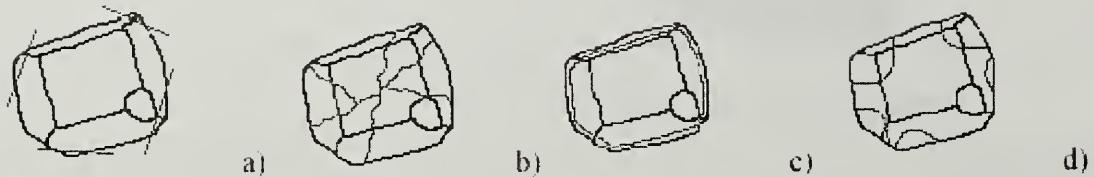


Figure 5: Different breakage mechanisms: a) attrition, b) fragmentation, c) abrasion, d) chipping.

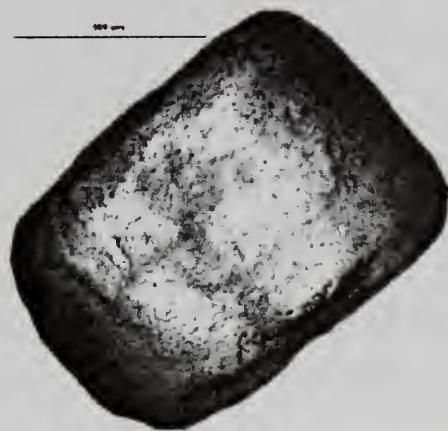


Figure 6 : Crystal abrasion

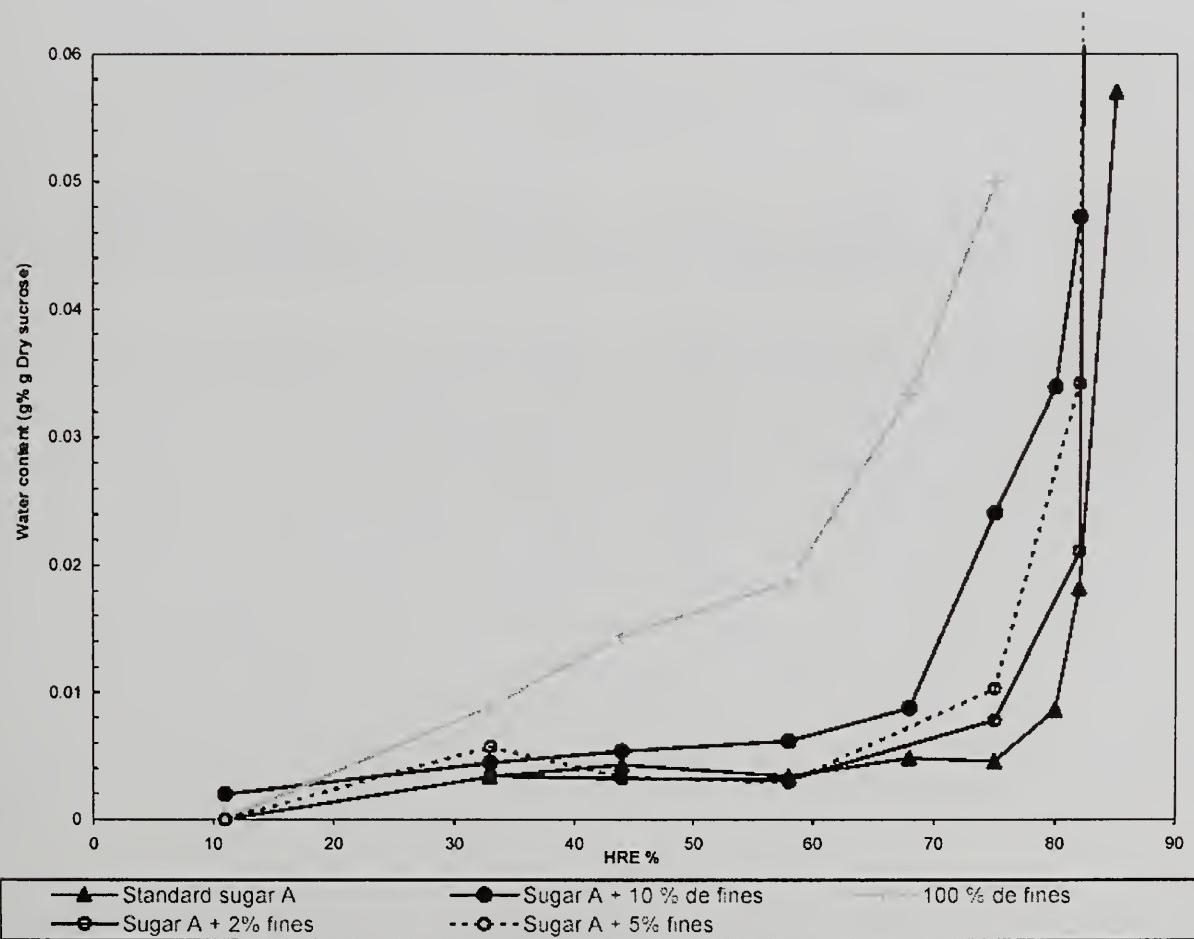


Figure 7 : Water vapor sorption isotherms of standard sucrose A, and A with added fine particles (2, 5 or 10 %) compared to water vapor adsorption of 100 % of fine particles (M.A. < 250 μ m)

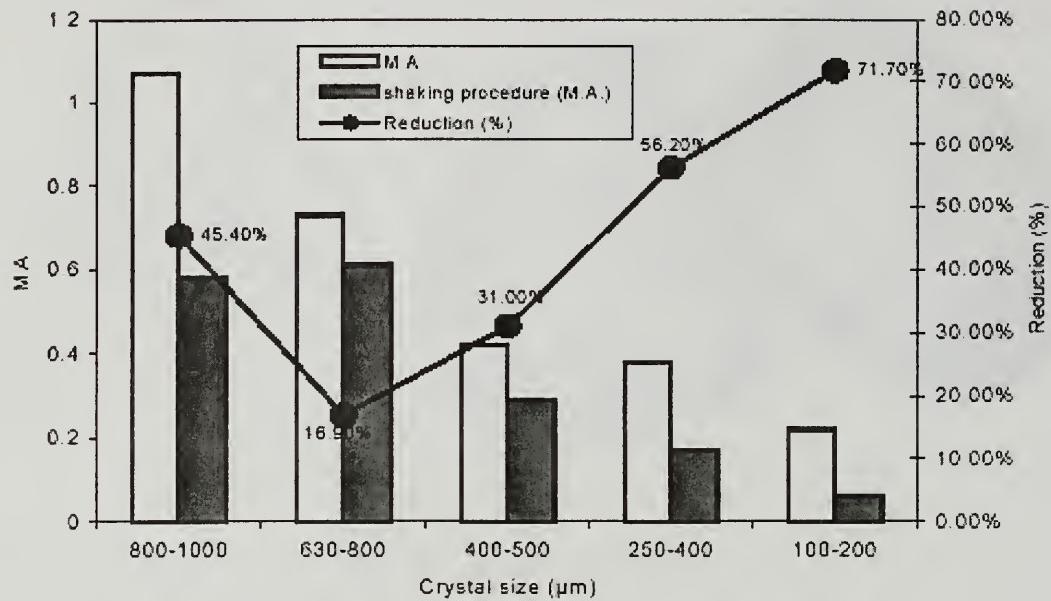


Figure 8 : Mean Aperture (M.A.) of different crystal size before and after a shaking procedure, reduction in % of mean aperture.

THE SUGAR INDUSTRY IN BETWEEN TRADITION AND THE FUTURE

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ABSTRACT

Sugar manufacture follows a traditional flow sheet: raw material (beet or cane), diffusion/extraction, raw juice purification, juice concentration, crystallization, sugar.

Such a scheme, even after moving to completely continuous processing, has remained unchanged in the course of the last more than 100 years. Important and fundamental the modernization which has taken place, and in particular as far as management, control and automation are concerned, but all involving single steps or procedures whilst the basic scheme remained unchanged. This fact permitted, and still permits, to limit the relevant investments not only going on with utilizing at the best already tested facilities and amortized plants but also even considerably enlarging factories by moving expensive machines from others, the closure of which has been planned. Bearing in mind this type of policy, which occurs in the presence of an unfavourable sugar market, it is understandable that the sugar industry is not open to proposals that, in one way or another, may upset the traditional trend of sugar manufacture. Some of these proposals, made in recent years, are considered and discussed and, in particular, the one, which is, more than a proposal, a reality, that is to bring new HIS, High Intensity Sweeteners, onto the market.

INTRODUCTION

At the opening Session of 21st General Assembly of C.I.T.S., held in Antwerp in May 1999, I presented a short review on the development of sugar technology over 50 years. I started my speech by showing two flow sheets of both beet and cane sugar manufacture with the aim of describing and commenting on what had been realized in the various stations of the sugar

factory, according to the course of processing from reception of the raw material up to the production of sugar.

Even though I will review here some of the more interesting and promising developments of sugar technology, which have already been realized in recent years, I would like to start from the same flow sheets which I utilized in Antwerp but considering them from a completely different point of view. In fact, both are based on the same principles: raw juice preparation, juice purification, juice concentration, crystallization, sugar, all processing steps which have remained unchanged for more than 100 years. I believe that some thoughts on the reasons for this immutability could be interesting and a subject for discussion.

PLANT UPDATING

As a matter of fact the process of sugar manufacture, concerning both cane and beet, has been regularly updated, but such updating has traditionally expanded, while maintaining its basic scheme. The first important step concerns the introduction of continuous processing when all the various steps of the process have been modified starting from the extraction/ diffusion plants up to the various types of continuous crystallization, although this latter change is based on seeds prepared separately. The various stations have been gradually equipped with the most sophisticated automation and control devices, which today permit not only supervision of the different phases of the processing, but even to programme them or to operate on-line. Dramatic changes have been carried out in beet handling, washing and water treatment, as well as the optimization of the raw material from its cultivation up to its harvesting and reception at the factory site.

Plants are constantly modified and improved, as well as having regularly updated automation, modern technology seeks the maximum energy saving and, due to the strict current regulations, the minimum environmental impact. In fact, the sugar industry takes care of the environmental problems and considerable funds are every day invested to solve environmental problems that, even a few years ago, were considered almost unimportant. These environmental problems even concern acoustic pollution as well as pollution caused by the raw material transport.

Another subject of great interest is processing safety and another one, no less important and current, deals with certification which concerns not only the final product characteristics but also others, which have seemed to be less important, concerning the factory and the adopted system of manufacture. The changes carried out on the various plants mentioned above involve even the materials utilized for their manufacture, so not only their reliability is guaranteed but also their maintenance costs are decreased with sensible advantage for the balance of the whole processing.

In both the beet and sugar end, research efforts are concentrated on the installation of increasingly improved equipment that will guarantee the best results such as, for instance, in the evaporator station. The goals achieved in thermal exchange, to be obtained with the maximum energy saving, concern the replacement of evaporators of the Roberts type with much more sophisticated plants employing falling film, plate-type falling film or rising film tubes which, on

the other hand, realize ever lower retention times. Moreover, as well as an appreciable decrease of the thick juice colour, steam economies can be achieved which can even reach 50%.

The use of ion exchange resins, which were mainly utilized for the thin juice demineralization, mentioned above, and others, which at the moment have not been mentioned, have has been considerably rescaled on account of the many problems related to their regeneration.

Even using molasses as the raw material for producing sugar has been considered by proposing processes that have been realized in various countries with different levels of success. Now different types of chromatographic plants permit the separation of sucrose from other compounds and, in particular, from betaine. The increased capacity of the factories from less than 1,000 to more than 13,000 tonnes of beet processed per day has greatly increased the daily production of sugar, and silos having sugar storage capacities of 40,000–50,000 tonnes or more are provided. Consequently, more and more sophisticated technologies for moving and storing sugar are sought, such as, for example, fluidized bed technology for cooling and drying. These modern technologies, which utilize very large columns containing ion exchange resins or sugar to be stored, involve problems of initial fluid distribution. These problems are solved also by adopting fractal distribution systems.

In order not to lose sight of the thread of my speech, I would like to point out that all the innovations not practically changed either of the two flow sheets shown above. The *spine* of the sugar factory has remained unchanged: beet, sugar diffusion, juice purification, concentration, crystallization, sugar or: sugar cane, sugar extraction, juice clarification, juice purification, concentration, crystallization, sugar. Everything which has been gradually installed has improved and optimized as much as possible the trend of the different stations but without completely replacing them and this point, which makes up the unchangeable tradition of the current sugar industry in the world, undoubtedly represents its strength. New plants and new equipment mean big investments. On the other hand, the technological, although very sophisticated, updating of existing facilities, which have shown over the years their rentability and which are operative and, most of all, at zero buying cost, are more accessible. The immutability of what I termed the *spine* of the sugar factory, has permitted and continues to permit great increases in the capacity of the factories via the recycling of a considerable portion of large and expensive basic plants from closed factories as well as to realize more reduced investment programmes.

“REVOLUTIONARY” PROPOSALS

Continuing research permits, as mentioned above, the realization of proposals able to make increasingly efficient and sophisticated the technologies existing and still operating inside the sugar factories. However, even proposals are presented which we could name “revolutionary” proposals that do not concern the change or replacement of particular machines or the utilization of new criteria for running a certain process in the course of the usual sugar factory processing. These are proposals that can radically modify or even eliminate some of the basic procedures which are fundamental steps of those flow sheets we are dealing with.

Such proposals, considered interesting from the beginning, even though they have successfully gotten through the experimental phases including pilot plant operation, can remain idle. Their study can go on at the R&D level and can even involve more than one country, thus collecting progressively new and interesting elements. However, the presently depressed sugar market does not permit the availability of any funding needed to carry out the final experiments on an industrial scale. In weighing-up counts the fact that already operating stations exist having plant which, as mentioned above, is already fully amortized and is not judged reasonably replaceable with others which have not yet obtained any confirmation on an industrial level.

As a matter of fact, since 1991 we have been involved with this type of policy when we undertook a study on cooling crystallization of raw beet juice with the aim of eliminating the whole juice purification from the flow sheet of sugar manufacture. This study continued in the last decade through continuous improvements, which fully confirmed the possibility of obtaining commercial white sugar directly from beet raw juice, which could then even undergo microfiltration or ultrafiltration and softening procedures for the further optimization of the results. Nevertheless, after having overcome, with the support of the sugar industry, laboratory tests and experiments at the pilot plant level, at the time of moving to industrial scale, the support of the sugar industry suddenly ceased.

Another interesting example of a proposal concerning the replacement in the cane sugar industry of the conventional purification, is based on the utilization of membrane micro- and ultra-filtration or even better, membrane nanofiltration. The utilization of the various types of membrane that the manufacturers put on the market would permit filtration procedures guaranteeing improvement of the raw sugar quality. Nevertheless, even if this is not a question of the elimination of the whole purification department but the replacement of the existing systems of conventional filtration equipment with others having better performances, the relevant benefits seem not to be such as to justify investment under the current economic situation. It seems that the various polymeric, inorganic or stainless steel membranes do not assure, at least at the moment, a reasonable lifetime as well as modes of failure so that their cost represents the largest portion of the operating expenses of any membrane system.

Furthermore, a group of researchers of the Department of CPRO-DLO in Wageningen, The Netherlands, published, in 1998, in *Nature Biotechnology* a paper, the content of which, even if not presented as a proposal, is undoubtedly revolutionary. The researchers introduced into sugar beet, by polyethylene glycol-mediated transformations of guard cells, the gene encoding 1-sucrose-sucrose fructosyl transferase from *Helianthus Tuberosus*. In the latter, 1-SST mediated the first steps in fructan synthesis through the conversion of sucrose into low molecular weight fructans, GF₂, GF₃ and GF₄. In roots of transformed sugar beet the stored sucrose would be almost completely converted into low molecular weight fructans. The authors point out that the phenotype would not be affected nor would the growth of the root, at least under greenhouse conditions.

Although bearing in mind that this investigation concerns a subject, that of GMOs, which is the object not only of a lively discussion but also of firm contention in many countries, the paper has raised great interest. Taking into account the merits of the oligosaccharides recently described by the nutritionists, it seemed possible that beet could be produced containing fructans instead of

sucrose. The container remains unchanged but the content changes, with all the advantages related to industrial utilization. Nevertheless, whilst research is going on in the field of transgenic beet and in particular on the breeding of roots resisting different types of herbicides and so on, the investigation mentioned above, at least at our knowledge, has been interrupted.

Always within the limits of our information, not only it seems that nothing has been accomplished in practice but no other papers have been published on the further developments of the research on both the laboratory or greenhouse levels.

Another proposal, if we term it as such, which can find its place among the ones we have named *revolutionary*, concerns the so-called High Intensity Sweeteners (HIS). The existence of alternative sweeteners is well known: the discovery of saccharin dates back to 1878, and this product has been on the market for more than 110 years in spite of its slight bitter, liquorice or metallic after-taste. Its sweetening intensity is approximately 300 times the intensity of sugar so that it is assumed that 1 tonne of saccharin has a potency of 300 times that of sugar, or 300 tonnes of sugar equivalent.

In the second half of the last century we find, among the others, Cyclamate which shows a sweetness intensity of approximately 35 times that of sucrose, Acesulfame K, approximately 200 times sweeter than sucrose, Aspartame approximately 200 times sweeter than sucrose and Stevioside as sweet as Acesulfame and Aspartame.

We find all these products on the market in competition with sugar but others are regularly manufactured, i.e. Hernandulcin, 1,000 times sweeter than sucrose, Monellin, 1,500-2,000 times sweeter than sucrose, Alitame, 2,000 times sweeter than sucrose and Thaumatin, 2,000-3,000 times sweeter than sugar. But the research goes on and other products are presented or even available on the market such as the Aspartame derivative Neotame, which is approximately 9,000 times sweeter than sugar and more stable than Aspartame. But new sweeteners are coming, either as such or as mixtures, which are 10,000-13,000 times sweeter than sugar.

This is a completely new scheme of things if we consider the concept of tonnes of sugar equivalent mentioned above. One tonne of the sweetest of the new sweeteners corresponds to 13,000 tonnes of sugar. In other words, as an example, 1,600,000 tonnes of sugar, the sugar consumption per year in Italy, corresponds to 123 tonnes of the new sweetener...and so on at a worldwide level. The hypothetical replacement of sugar from HIS would obviously involve not only the sugar as such, but also some other sweeteners having lower sweetness intensity and in particular even HFCS which, in the last decades, has been very successful, in particular in the USA.

CONCLUSIONS

At the beginning of this presentation, I started by discussing the two flow sheets that are the basis of sugar manufacture either from beet or cane, pointing out that they can be considered the strength of the sugar industry in the world. We have observed that the defense of these traditional schemes against proposals able to change them can be justified by the utilization of factories that are not only still efficient but already amortized. An essential role is also played by the

possibility of introducing into the existing flow sheets all the modern and sophisticated automation systems that continuously update their performance and control.

Even if in the immediate future, depending upon the situation of the sugar market, some of the revolutionary proposals mentioned above (cooling crystallization, membrane micro-, ultra- or nanofiltration) can find significant application, a special comment is due to the success of the HIS taking into account that, at least to our knowledge, no other information has appeared in the recent literature on *fructan-beet*.

The order of magnitude of the sweetness intensity of Neotame and its availability on the American market this year, after its approval in the USA by the FDA in June 2003, could open upsetting horizons, suggesting new initiatives to the suppliers of potent sweeteners who could plan to develop their markets at sugar's expense.

Nevertheless, many points remain in favour of tradition, first of all the fact that sugar consumption is steadily on the rise. We have not to forget that sugar provides bulk, assists in gel formation, adjusts water activity, contributes to browning, to name only a few points, and that, lacking these characteristics, HIS cannot be used alone.

Moreover, although a certain number of HIS are in the pipeline for approval, before obtaining such approval, these sweeteners, which are defined by many governments as *food additives*, must be submitted to stringent safety evaluation by independent expert committees. The extremely high costs involved in the legally required safety evaluation and the length of the evaluation and approval procedures certainly constitute a barrier that can be very difficult to overcome. Moreover, to recover the expenses for the introduction of a new sweetener on the market, it would be necessary from the HIS manufacturers to obtain a considerable market share within a reasonably short period of time.

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ETHANOL PRODUCTION BY THE ZEACHEM PROCESS: AN ELEMENT OF A SUGARCANE BIOREFINERY

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ABSTRACT

In the near future the biorefinery concept of complete utilization of cane field biomass will become a pivotal element for a sustainable sugarcane industry. An industry that produces liquid fuels, electricity and commodity chemicals from a renewable source in regional communities will contribute to all elements of the triple bottom line and should be considered a compelling vision. In Australia, the Cooperative Research Centre for Sugar Industry Innovation through Biotechnology commenced in August 2003 and will embark on a significant program of research and development to underpin sugar cane biorefining. Fermentation to ethanol and other renewable commodities and biomass treatment processes such as pulping are technical elements of the biorefinery concept. The Sugar Research Institute has undertaken to assess the ZeaChem process – a novel fermentation route to bio-ethanol. This presentation covers the capacity of the Australian sugar industry to produce ethanol and our preliminary assessment of the ZeaChem process.

The Capacity of the Australian Sugar Industry to Produce Ethanol

The capacity of the Australian sugar industry to produce ethanol has been assessed by Bullock [1] and this assessment has been referred to extensively in a number of recent documents, for example in the Australian Bureau of Agricultural and Resource Economics report titled ‘Viability of sugar cane based fuel ethanol’ [2]. The Australian sugar industry currently produces ca. 60 megalitres of ethanol per annum. This ethanol is produced entirely from final molasses (or C molasses). If the entire industry’s production of C molasses was directed towards ethanol production, then ca. 280 megalitres could be produced. If B molasses (an intermediate

product with higher fermentables) was directed to ethanol production (as is the case in the Brazilian sugar industry), then ca. 750 megalitres of ethanol could be produced.

The ZeaChem process (discussed below) has the potential to increase ethanol yield from sugar cane feedstocks by ca. 60 %. If the entire industry's production of C molasses was directed towards ethanol production by the ZeaChem process, then ca. 450 megalitres could be produced. If B molasses was directed to ethanol production, then ca. 1200 megalitres could be produced.

A Preliminary Assessment of the ZeaChem Process

The ZeaChem process is a novel approach to the production of ethanol from corn dextrose that utilises a combination of known fermentation and chemical synthesis steps. ZeaChem Inc., founded as a partnership in 1998 and reorganized as a corporation in 2002, has one international patent [3] and several provisional patent filings. The Sugar Research Institute (SRI) has reached an agreement with ZeaChem Inc. to jointly develop the ZeaChem technology for conversion of sugarcane processing streams to ethanol.

Existing technologies for ethanol production rely on direct fermentation of carbohydrates derived from corn, sugarcane and other sources. All direct fermentation routes suffer from low carbon efficiency.

Conventional Fermentation: $\text{Glucose} \rightarrow 2 \text{ Ethanol} + 2 \text{ Carbon dioxide}$

In conventional fermentation two of the six carbon atoms in the substrate are converted to carbon dioxide, giving a maximum carbon efficiency of only 67%. On a mass basis, the maximum obtainable yield of ethanol from six-carbon sugar is only 51%, with the remaining 49% being converted to carbon dioxide.

The ZeaChem process avoids the problem of low carbon efficiency by using an indirect route based on a combination of fermentation and chemical syntheses steps. Carbohydrates are converted to acetic acid by one or two fermentation steps, acetic acid is esterified to ethyl acetate, and ethyl acetate is hydrogenated to ethanol.

Net ZeaChem Process: $\text{Glucose} + 6 \text{ Hydrogen (gas H}_2\text{)} \rightarrow 3 \text{ Ethanol} + 3 \text{ Water}$

Ethanol production by the ZeaChem process results in a 50% yield improvement over conventional technologies (i.e. 3 moles of ethanol per mole of six carbon sugar instead of 2 moles of ethanol per mole of six carbon sugar). None of the fermentable carbohydrate is converted to carbon dioxide.

The ZeaChem patent [3] consists of two embodiments for the conversion of carbohydrate to ethanol. The first embodiment is fermentative conversion of the carbohydrate source to lactic acid and single cell protein (SCP), with subsequent fermentative conversion of lactic acid to acetic acid, esterification and hydrogenation. The second embodiment is direct fermentative conversion of carbohydrate to acetic acid. The first embodiment has the economic advantage of

producing SPC, a valuable by-product, but the disadvantage of being a two step process (compounding less than theoretical yields).

Stoichiometry of the ZeaChem Process

The ZeaChem process can be represented by the following three reaction equations:

1. Bacterial fermentation of carbohydrate to form acetic acid:



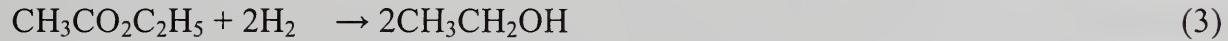
It is recognised that other substrates are also fermentable namely:

- C3: glycerol, lactate
- C5: xylose
- C6: glucose, fructose
- C12: sucrose, lactose, cellobiose, maltose

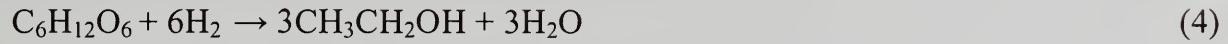
2. Esterification of acetic acid with ethanol to form ethyl acetate:



3. Hydrogenation of ethyl acetate to form ethanol:



The overall process can be represented by:



Production of Acetic Acid by Bacterial Fermentation of Sugarcane Carbohydrate Feedstocks

The acetogenic bacteria that carry out the fermentation step are well studied [4]. Homoacetate fermentations are characterised by formation of acetate as the sole end product and were discovered by Fontaine in 1942 [5,6]. They are gram-negative obligate anaerobes with the unique capability of metabolizing many substrates into acetate at near 100% theoretical carbon efficiency. Readily converted substrates include lactate, five carbon sugars including xylose, six carbon sugars such as glucose, and disaccharides such as sucrose. Demonstrated laboratory yields typically range from 85 to nearly 100% of theoretical with final acetate concentrations of 30 to 40 g/l [7-12]. Optimal pH is usually near 7, so neutralization of the broth with a base such as calcium carbonate or lime is required.

Of the homofermentative acetogens, *Clostridium thermoaceticum* is most commonly evaluated for the biological production of acetic acid [e.g. 7-15]. The main limitations of this bacterium are that it suffers from product inhibition (~5 % acetic acid in fermentation broth) and it has extensive nutrient requirements. In order to produce acetic acid on an industrial scale a low cost media is required and low yields of acetic acid (due to product inhibition) influence the economics of product recovery [10].

Clostridium thermoaceticum ferments glucose with the formation of ~3 mol of acetate per mole of glucose. Two acetate molecules are formed by an Embden-Meyerhoff type cleavage of glucose to pyruvate followed by oxidation of the pyruvate to acetic acid and carbon dioxide (using regular oxidation pathways). The third acetate is formed by reduction of carbon dioxide (acetogenic hydrogenation) [4,5].

Clostridium thermoaceticum can ferment a variety of substrates including glycerol, lactate, xylose, glucose, fructose, sucrose, lactose, cellobiose, maltose and higher dextrans. It has also been shown that *C. thermoaceticum* can utilise acid-hydrolysed lignocellulose (e.g. hydrolysed agricultural wastes, bagasse), which contain organic acids and aromatic compounds such as phenolic acids derived from lignin [14].

The optimum conditions for growth are:

Temperature: 60 °C

Carbohydrate concentration: ca. 20 g/l to avoid product inhibition

Trace elements requirements: nickel, selenite, tungstate, molybdate and iron-sulfur clusters.

Nickel is a component of carbon monoxide dehydrogenase in *C. thermoaceticum*. The production of the NADP-linked formate dehydrogenase in *C. thermoaceticum* depends on the presence of selenite, tungstate, and molybdate in the growth medium. Iron-sulfur clusters (4Fe-4S) are redox active and maintain a reducing environment. Acetate ions inhibit growth (product inhibition) and to a much lesser extent growth may also be inhibited by the presence of chloride, sulfate, ammonium, potassium and sodium.

The compositions of sugar processing streams are complex. Each stream (e.g. secondary juice, mixed juice and the molasses) will need to be evaluated as carbohydrate sources for acetic acid production. For this evaluation, three strains of *Clostridium thermoaceticum* have been identified, viz. DSM 521 (German Culture Collection), ATCC 39073 and ATCC 49707 (both from the American Type Culture Collection). These cultures have been obtained and fermentation trials are underway. These trials include shaken flask, stirred batch and stirred fed-batch experiments.

Esterification of Acetic Acid and Ethanol by Reactive Distillation

Reactive distillation combines chemical reaction and distillation into a single process step. Saito et al. [16], Shoemaker and Jones [17], Agreda et al. [18], Doherty and Buzad [19], Kenig et.al [20,21] and Mehrabani [22] described the advantages of reactive distillation in industrial applications. In some applications and particularly when thermodynamic reaction equilibrium prevents high conversion efficiency, the coupling of distillation to reaction removes the reaction

products from the reaction zone and significantly improves the overall conversion and selectivity. In other applications, reactions are utilised to overcome the separation problems caused by azeotropes. This combination of reaction and distillation often results in simple intensified processes, with less recycle streams. This reduction in recycling should effect a reduction in capital and operating costs.

Reactive distillation is fast becoming a preferred alternative to conventional reaction and separation processes. The increasing importance of this process is manifest in the growing number of research publications and for example, Eastman Chemical operates a reactive distillation system that produces 400 MM lb/yr of methyl acetate using methanol and acetic acid as feedstocks [18].

While conventional ethyl acetate production utilises traditional reactor designs [23], reactive distillation may be used for ethyl acetate production. In fact in research literature on reactive distillation, esterification of acetic acid and ethanol to water and ethyl acetate is the most frequently considered reactive system [20-22,24-30]. Esterification conditions can be selected so that yields close to 100% of theoretical are achieved. Unfortunately the reaction proceeds smoothly only when the acetic species is present in its acid form rather than as an acetate salt.

In the esterification of *C. thermoaceticum* fermentation products, a mineral acid could be used for acidification but this option would produce a salt by-product. Alternatively, acidification can be achieved with carbon dioxide and an extractive purification system that uses a regenerable amine to complex with the acidified components of the fermentation broth [24]. This second method gives yields in excess of 90%, does not produce a salt by-product and has the additional advantage of concentrating the acetic acid prior to esterification. This concentration step will lower energy requirements for recovery and alleviate concerns over the relatively dilute acetate concentration produced by the fermentation step.

Hydrogenation of Ethyl Acetate to Ethanol

Hydrogenation of esters to produce alcohols is a well-known organic reaction [31] that has been used commercially to produce products such as 1,4-butanediol and ethylene glycol. The reaction can be done either in the gas phase or the liquid phase using readily available commercial hydrogenation catalysts based on copper chromite, Raney nickel and several other formulations. Typical gas phase conditions for hydrogenation of ethyl acetate are 200°C at 500 psi, giving single pass yields of about 80% [32,33]. Since the selectivity is close to 100%, recycling can drive overall yields to near 100%.

The hydrogen used in the ZeaChem process is a significant energy source. It contains ~30% of the energy content of the final ethanol product. There are many potential hydrogen sources and any process producing hydrogen of sufficient purity for the hydrogenation can be used. The ZeaChem patent refers to electrolysis of water, biomass gasification, steam reforming natural gas and other process based on fossil fuel feedstocks. The costs of various sources of hydrogen have been assessed in terms of contribution to the cost of ethanol in the ZeaChem process. Data from Padro and Putsche [34] was converted into Australian dollars and in the case of hydrogen from

reforming natural gas the cost of gas was adjusted to the Australian cost (so that it would be directly comparable to our analysis of the cost of hydrogen production at Phosphate Hill). The analysis assumed a 90% conversion efficiency in the hydrogenation reaction (i.e. 10% of H₂ lost in process). The results of this costing assessment are shown in Figure 1. We are also aware of the possibility of producing hydrogen by catalytic reforming in liquid water [35-37] and by the reaction of biomass with molten iron and tin [38]. These are experimental processes and the costs of hydrogen from these processes have not been determined.

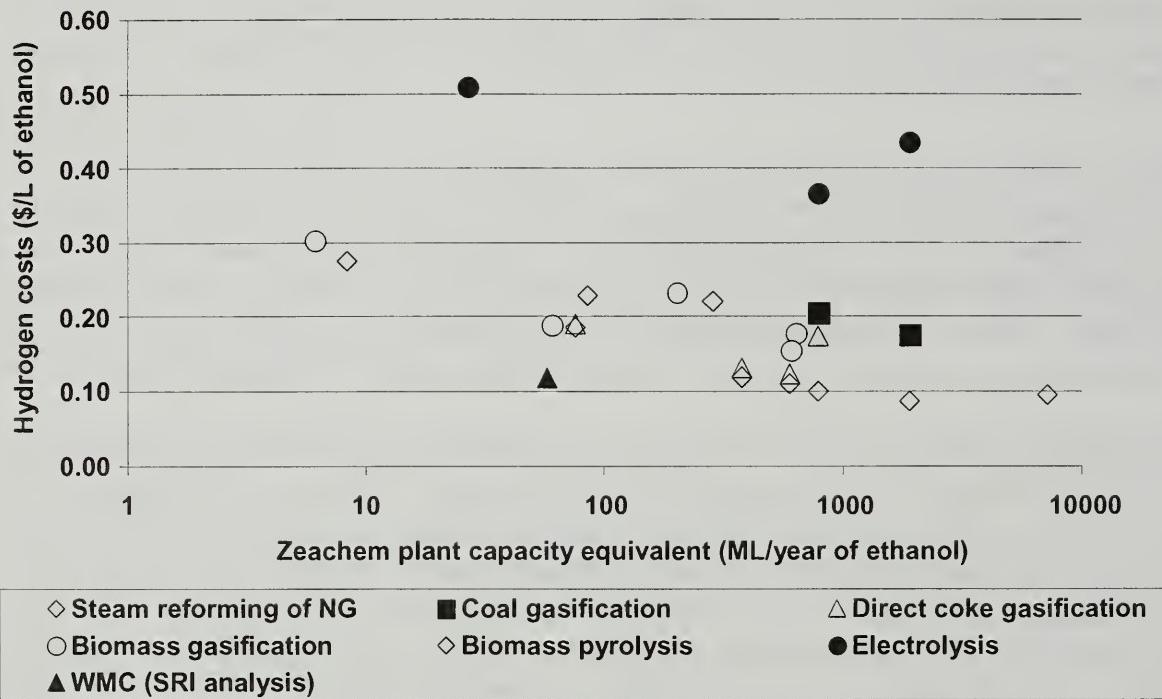


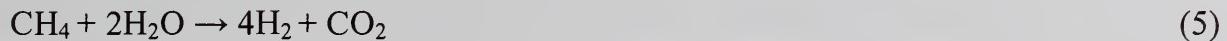
Figure 1. Assessment of the cost of hydrogen from various sources.

By far, steam reforming of natural gas is the cheapest source of hydrogen. However, while steam reforming of methane is a low capital cost option that uses well proven commercial technology, it is non-renewable. Consequently, we have also taken an interest in the Kvaerner process for producing hydrogen and carbon black from methane and the use of coal seam methane as these options have significant influence on life cycle analysis outcomes.

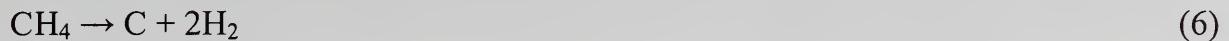
Preliminary Mass and Energy Balance Analysis on the ZeaChem Reactions

A simple mass and energy model of the coupled ZeaChem reactions has been implemented in HYSYS.Process, a process flow simulation code. HYSYS.Process is a commercially available general-purpose code (developed initially for the petrochemical industry) with the capability of simulating material and energy flows in a wide range of unit operations under both steady state and transient conditions. It is anticipated that this code will be used almost exclusively in the subsequent detailed design and costing phases of the current project.

In addition to the ZeaChem reactions (equations 1 to 3), two processes to produce hydrogen have been simulated, these being the steam reforming of natural gas (the latter assumed to be predominantly CH₄):



and the Kvaerner process (also using natural gas as the feedstock) for producing H₂ and carbon black given by:



For the purposes of this preliminary study, the HYSYS model has been set up with the following primary assumptions:

- Heat and mass flow are steady state
- Reactions proceed to a prescribed state of completion rather than to a state of completion determined by equilibrium conditions or reaction rates. This assumption was imposed simply as a mechanism by which the impact of individual reaction levels on overall ethanol yield could be readily determined.
- Reaction energies have been determined assuming isothermal conditions are maintained. That is reactants are assumed to be initially at 25 °C and the heat of reaction is determined as the total heat released as the products are returned to this initial temperature. This standard approach was adopted so that the thermodynamics of the chemical processes would not be swamped by issues relating to heat transfer. The latter are highly technology dependent; choice and configuration of equipment strongly influences sensible and latent heat recovery and therefore energy efficiency.
- In the event that a reaction does not go to completion, residual reactants are subsequently removed from the process rather than continuing through to the next reaction. Given that only prescribed chemistry would occur in given reactor vessels in the model, this assumption has no effect on subsequent reactions. The assumption has been implemented to facilitate the auditing of un-reacted feedstocks and the numerical stability of the calculations.
- Physical and chemical properties for all reactants (with the exception of dextrose) and products are extracted from an extensive set of HYSYS data libraries. A comparison of these HYSYS generated property values with standard texts shows good general agreement. Where property values are not available within HYSYS, the program calculates approximations based on inputted molecular composition and basic physical properties such as density and boiling point. This latter process gave poor estimates for the heat of formation of dextrose and a value of -1270.7 kJ/mol was manually entered into the HYSYS property sheet.

This analysis indicates that with the exception of the steam reforming reaction, all reactions are exothermic. The summed energies from these exothermic reactions and the endothermic steam reforming reaction indicate that the whole process is weakly exothermic (-65 kJ/L of ethanol) with 100% conversion efficiency for all reactions. In reality the conversion rate of the hydrogenation reaction will significantly influence the energy balance. As the conversion rate of the hydrogenation drops, the hydrogen demand per litre of ethanol will rise as will the energy demand of the endothermic steam reforming process. This effect is shown in Figure 2 where to put things in context, the overall energy demand of the ZeaChem process is expressed as a percentage of the fuel value of ethanol produced. A Lower Heating Value for ethanol of 26,750 kJ/kg or 21,069 kJ/L is used in Figure 2.

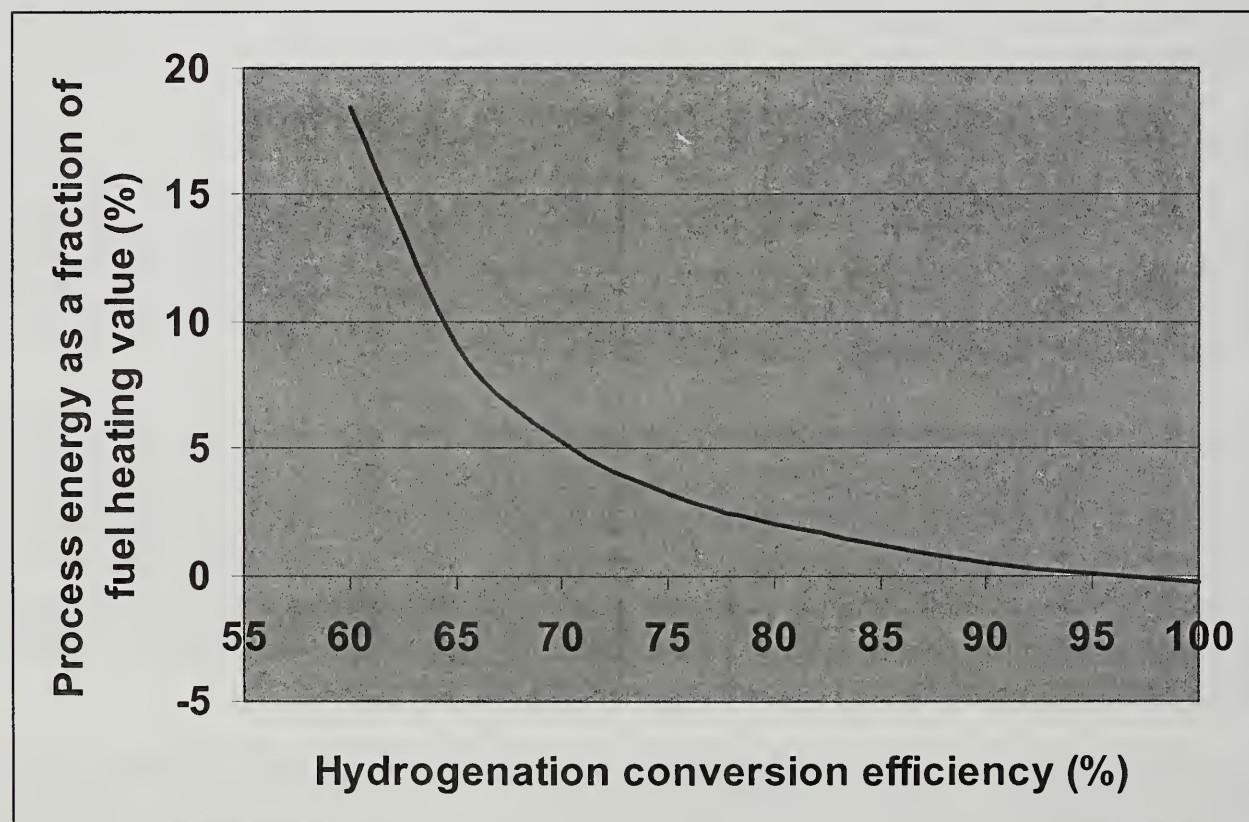


Figure 2. Variation of net energy consumption with hydrogenation reaction efficiency

CONCLUSIONS

The ZeaChem process appears to be technically feasible and when practiced in industry will significantly increase the capacity of the Australian sugar industry to produce ethanol.

ACKNOWLEDGEMENTS

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Dr Graeme Bullock. The ZeaChem assessment project is funded by Agriculture Fisheries and Forestry Australia, the Queensland Government Department of State Development and Sugar Research and Development Corporation.

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A NEW TECHNOLOGY FOR REGENERATING SUGAR DECOLORIZING ACTIVATED CARBON

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Foreword by Dr. Amos Turk, Emeritus Professor of Chemistry, CCNY

After nearly half a century in Academia studying the applications of activated carbon to real world applications, I have come to appreciate that activated carbon is easier to utilize than to understand. Dr. McLaughlin has undertaken to demystify some of the phenomena observed with activated carbon adsorption and regeneration. While much of what appears in his discussion is not common knowledge, it does represent the current thinking on the inner workings of activated carbon.

Dr. McLaughlin's current paper first develops a coherent framework for understanding activated carbon, and then delves into the low temperature oxidation of activated carbon in air. The conditions in which such oxidation occurs are not well understood and the phenomenon has been unrecognized by the commercial activated carbon industry. McLaughlin has identified the synergism of the slow oxidation of the granular activated carbon and the concurrent and unexpected preferential oxidation of the adsorbed organics. Such a phenomenon provides a novel and attractive method for regenerating spent activated carbon.

After 50 years, it is good to see that there are still new ideas, applications, and regeneration technologies being developed for activated carbon.

INTRODUCTION

The essence of sugar refining involves removing the non-sucrose impurities, which include both particulates and soluble contaminants, followed by crystallization of product from the mother liquor and packaging. While solid-liquid separation processes such as filtration and clarification remove the largest mass of impurities, the final purification is done by physical adsorption on a

carbon-based material. Historically bone char provided this final purification, with modern industrial options featuring granular activated carbon, powdered activated carbon and ion exchange resin-based processes.

As summarized in the 11th edition of the Cane Sugar Handbook:

Decolorization is the key process in refining sugar. Of all the differences between raw and refined sugar, color is the one property that is immediately obvious and can be easily measured.

When a sugar chemist says “color” he means “colorant”, the material causing the color.

The decolorization process actually removes more than color because colorants interact with color precursors, colloidal materials, organic non-sugars, and ash-forming inorganic constituents so they are taken out with the color. (1)

While it often goes under-appreciated, decolorization really encompasses the removal of spurious odor and taste compounds, in addition to visible color. Decolorization renders the granulated sucrose stable for long-term storage and reliably reproducible in a wide variety of foods and cooking applications. The world enjoys and demands very high quality refined sugar.

As noted above, the ultimate purification step in sugar refining is physical adsorption of the color bodies onto a carbon-based adsorbent, typically activated carbon or ion exchange resin. Physical adsorption implies that the color bodies transfer from the bulk solution into the internal surfaces of the adsorbent, but the color bodies remain chemically unchanged. While the effect of the adsorbent on the sugar solutions is conceptually straightforward, in that unwanted compounds are removed from the aqueous sucrose solution, the impacts and consequences of the adsorption step on the adsorbent are considerably more complex.

Once the adsorbent has accumulated its capacity for adsorbable compounds, it is termed “spent” and must be replaced with fresh adsorbent. Exiting the adsorption process, after sweetening-off, any spent adsorbent consists mainly of the original adsorbent and the accumulated color bodies.

One option for spent adsorbent is disposal after a single adsorption cycle. This is the typical industrial practice when using powdered activated carbon. While powdered activated carbon is probably the least expensive adsorbent to purchase on a unit-weight basis, disposal after a single decolorizing cycle generally results in higher operating costs than other adsorption media which can be regenerated and reused. Furthermore, the spent powdered media with color bodies represents a continuing environmental concern, since the color bodies are subject to biological degradation, which can lead to odor issues and water contamination concerns.

For granular activated carbon and ion exchange resins, regeneration and reuse is the industrial norm. It should be noted that regeneration speaks to the impact on the adsorbent – recovering some or all of the adsorption capacity – and does not specify the impact on the previously adsorbed color bodies.

In the case of ion exchange resins, the regeneration process desorbs the color bodies into another aqueous solution – and the disposal of this “spent regenerant” solution must be addressed. Depending on the local circumstances, the outlet may be a POTW (Publicly Owned Treatment Works), a dedicated on-site treatment capability or discharge untreated into receiving waters. While the later practice may be exceptionally cost-effective, the deterioration of the local waterways is often undeniable and unacceptable over the long term.

One study (2) looked at the option of treating spent ion exchange regenerant brines with granular activated carbon, with the majority of the brine being recycled and upwards of 10 percent wasted after removal of the carbon-adsorbable and biologically-degradable fraction. While this approach appeared technically viable, the merits of removing color bodies with ion exchange resin, then removing them from the resin only to adsorb them back onto granular activated carbon seems like a circuitous route compared to adsorbing the color bodies with activated carbon in the first place. However, the study was basically driven by the desire to reduce regenerant brine consumption and avoid disposal of high BOD spent brine. As such, the study was a success, although the practice does not seem to enjoy widespread industrial popularity.

When granular activated carbon is utilized for color removal, the subsequent regeneration process typically results in the destruction of the color bodies. The most prevalent industrial practice is termed “high-temperature reactivation” (HTR) and is usually performed in multiple hearth furnaces or rotary kilns. The process is called “reactivation” because the regeneration conditions mirror the original conditions utilized during the manufacture of the virgin activated carbon in the “activation” step.

Another option for the regeneration of spent granular activated carbon is termed “low-temperature regeneration” (LTR). This approach is a recently developed option that appears to have significant advantages over traditional “high-temperature reactivation” and these features will be explored later in this paper.

In order to explore the technical and operational considerations involving the regeneration of spent granular activated carbon, it is necessary to develop a framework for evaluating the performance of a regenerable and reusable adsorbent. After establishing an understanding of how granular activated carbon adsorbents are utilized in the real world application of sugar decolorization, the performance advantages of low-temperature regeneration over high-temperature reactivation will be discussed.

Understanding and Evaluating the Performance of Recycled Adsorbents

In practice, the granular activated carbon adsorbent is recycled to “extinction”. This means that the entire mass of spent adsorbent is regenerated and returned to adsorption service. Virgin activated carbon, termed “make up”, is added to the regenerated material to maintain a constant volume or inventory of adsorbent.

Figure 1 (3) identifies several of the common causes of mechanical attrition of the granular activated carbon particles as they circulate between adsorption and reactivation service. It is noteworthy that only one percent of the losses are attributed to the gasification conditions in the

multiple hearth furnace. Higher carbon losses in the furnace are ascribed to oxygen leaks due to air infiltration, which combust available carbon until the oxygen is consumed.

This partitioning of blame for carbon losses is not entirely fair for reasons that will be explored later in the paper. Briefly, the high-temperature reactivation conditions cause a gradual but progressive weakening of the mechanical strength of the individual activated carbon particles. Eventually, this weakening causes the particle to collapse into fines during transport to and from the adsorption service. Thus, while the location of the fines generation is in the transport piping, the root cause is the effect of the high-temperature reactivation on the graphitic backbone of the activated carbon particles.

Before delving into the specific differences in the regeneration options, it is useful to understand the interactions that occur between the effectiveness of the regeneration process and the make up rate of virgin activated carbon. The current industrial practice is to add virgin carbon to “make up” the amount of carbon in circulation to a constant volume, in order to keep the adsorbers full during the decolorization process. Hence, there is a tendency to consider the rate of virgin carbon addition to be a measure of the total losses that occur during the regeneration process.

Unfortunately, another significant loss during regeneration is the loss of adsorption capacity per unit volume of carbon. This loss is much harder to evaluate and generally is not measured during the routine operation of a carbon adsorption system. However, if this capacity loss were not occurring, then the “pool” of circulating carbon would have the same adsorption capacity per unit volume as virgin activated carbon – which is simply not observed in actual practice.

The circulating carbon pool generally exhibits 50% to 75% of virgin activated carbon capacity – based on testing of samples of industrial pool carbons. This loss of capacity phenomenon is not well documented in the literature, although it is discussed in an older article comparing the performance of bituminous coal carbons to lignite carbons (4).

The capacity loss per cycle for an individual carbon particle depends on many variables, as discussed in Reference (4). However, since the pool carbon consists of carbons added as make up over many cycles, the overall effect is a relatively constant loss of adsorption capacity per adsorption-regeneration cycle (due to the averaging of the different age carbons present).

This behavior can be modeled for a given circulating pool of activated carbon. One first assumes a prescribed loss of adsorption capacity per regeneration cycle. Then, one reduces the amount of material per cycle by the make up rate and then adds back of the same amount of virgin carbon. Repeating the process calculates the evolution of the adsorption capacity of the pool carbon for each regeneration cycle. By numerically calculating the effect of a large number of iterations, the steady-state properties of the pool carbon can be calculated. The results of such a simulation are shown in Figure 2.

Figure 2 shows some very interesting trends. For the hypothetical “perfect” regeneration, where the regenerated carbon is returned to virgin carbon adsorption capacity or a capacity loss of 0% per cycle, the pool capacity remains at 100% of virgin, independent of make up rate. For any significant capacity losses, 2% per cycle or more, the pool capacity is significantly less than

virgin and very sensitive to make up rate. For any non-zero capacity loss per cycle, the greater the make up, the greater the pool capacity, since the make up carbon is virgin carbon with 100% capacity.

One real world situation that demonstrates how sensitive the pool capacity is to the combination of capacity loss and make up rate is the case of under-reactivation in the multiple hearth due to insufficient steam. The effect is to regenerate with a higher capacity loss, since char is left in the carbon (as will be discussed later), but reduce the amount of make up carbon necessary. For the case of low steaming rates, it is possible to have 6% capacity loss per cycle and 4% make up rate. In contrast, higher steaming rates would likely produce 4% capacity loss per cycle, but require 6% make up rates.

As shown on Figure 2, the lower steaming rate and associated 6% per regeneration capacity loss results in a pool capacity of only 40% of virgin carbon capacity; whereas the higher steaming rate results in 60% of virgin capacity. Thus, the higher steaming rate yields regenerated carbon with 50% higher adsorption capacity per adsorption cycle, which directly translates into the amount of sugar refined before the carbon becomes spent and must be regenerated.

One straightforward but computationally complex exercise is to optimize the performance of the pool carbon based on the incremental cost of reactivation, make up carbon, and operating costs for the adsorption cycle. Such analyses are unique to each individual industrial site, since the operating costs are site specific, but yield great insight into the preferred operating practices of both the adsorbers and the reactivation facilities. It is not unrealistic to identify a 25% reduction in direct operating costs of the combined systems based on revising the operating setpoints of an existing high temperature reactivation facility.

Measuring the Adsorption Properties of Activated Carbon

Granular activated carbon is a unique material that combines intrinsic properties of naturally occurring starting materials and aggressive activation conditions to create a micro-porous graphitic lattice that possesses exceptional adsorption properties. The adsorption properties are largely due to the polarizability of the localized graphitic structures, which results in "London dispersion forces" that facilitate the pure physical adsorption within the internal surface area of the activated carbon.

In liquid phase adsorption, individual chemical groups on the surface of the graphite plates can also play a role in the adsorption equilibrium by contributing hydrogen bonding to polar adsorbates. The most significant variable is the degree of oxidation of the carbon surface, which can feature virtually every type of organic functional group. However, the dominant characteristic that determines adsorption performance remains the distribution of adsorption sites within the activated carbon.

The major starting materials for activated carbons are bituminous coal, coconut shells and hardwood sawdust. Each starting material imparts mechanical and adsorptive qualities to the finished activated carbon. In addition, the extent of activation allows a given starting material to produce a spectrum of activated carbons optimal for a variety of adsorption applications.

Figures 3a, 3b and 3c (3) show graphical representations of activated carbons produced from the three major starting materials. Figure 3a depicts coconut-based carbon, which features highly developed micropores that favor gas phase adsorption applications. Coconut shell carbons were the earliest industrial activated carbons. Only when the Japanese threatened the Allied supply of coconut-based material was a wartime research effort undertaken to manufacture activated carbon from coal feedstocks. However, coconut-based carbons still have properties that cannot be duplicated in coal-based carbons.

Figure 3b depicts wood-based carbon, with much large larger portion of transport pores and proportionally less carbon skeleton. The net result is much higher adsorption capacity at significantly lower adsorption energies, which favors desorption of weakly bound adsorbates. These properties result in the dominance of wood-based carbon in solvent recovery systems using direct steaming and temperature-swing as the desorbing method. Unfortunately, wood-based carbons lack mechanical strength, which often precludes high-temperature reactivation of spent wood-based carbons that have accumulated higher boiling VOC's.

Figure 3c depicts coal-based activated carbon. Coal-based carbons have come to dominate virgin activated carbon production – principally because it is much easier to get a ton of coal than a ton of coconut shells. Coal-based carbons have good mechanical strength and adsorption properties intermediate between coconut and wood-based products. The principal concern in coal-based carbons is higher ash levels, which can be controlled during manufacturing or modified by acid washing the final product.

Modern production of activated carbon provides the ability to specify the overall particle size and shape and enhance the internal transport properties through a technique called “reagglomeration”, where the starting material is pulverized and reassembled with binder. Coupled with the ability to vary the extent of activation of a starting material to create a spectrum of products, the current offering of virgin activated carbon products is literally in the hundreds.

With such flexibility in manufacturing, the properties of the virgin carbon can be optimized for a given adsorption application. The challenge in an industrial application, such as sugar decolorization, is to keep the activated carbon operating at or near optimal performance. This goal is even more difficult in a recirculating pool of carbon, where carbons of differing ages are present. “Age” in the context of a recirculating carbon system refers to the number of adsorption-regeneration cycles that an individual carbon particle has experienced.

The key to controlling the regeneration of “pool” carbon is to be able to assay the regenerated carbon in a manner that correlates with and distinguishes the subsequent adsorption performance. Unfortunately, such a task is easier said than done.

As a conceptual starting point, envision the internal structure of activated carbon filled with an adsorbed vapor. Vapor phase adsorption is often described as forming regions of liquid-like adsorbed material in equilibrium with the concentration of adsorbable chemical in the vapor phase. Figure 4 (3) depicts the carbon structure of Figure 3c for the case of butane adsorption from a stream of pure butane at room temperature.

Under such conditions, much of the micropore structure is flooded with liquid-like adsorbed butane, but some regions have much higher adsorption forces associated with the adsorbed butane. A broad range of adsorption sites that form a distribution of adsorption energies characterizes all adsorption applications using activated carbon. The challenge is to measure this distribution under conditions that provide meaningful insight to the industrial application one is striving to understand and control.

Figure 5 (3) tabulates the mainstream analytical methods that focus on carbon adsorption performance. Many of the tests are ASTM procedures that were developed many years ago. Keeping in mind that activated carbon has a broad distribution of sites of differing adsorption energies, one needs to appreciate the potential shortcomings of attempting to characterize this distribution with only one or two data points. However, if a single data point or two accurately distinguishes good performance from bad, then that is sufficient to make the distinction. If the data points cannot tell the difference, then they are of limited, if any, value.

Most of the historical single point measures listed in Figure 5 assay total pore volume, which is basically the maximum adsorption capacity, without any real distinction of adsorption energies. The only test that aspires to simulate actual adsorption selectively is the Molasses Decolorizing Number. Unfortunately, this test is tedious and very difficult to assign any real meaning to the value of the number obtained. As such, Molasses Decolorizing Number is often measured, but almost never used in a decision-making capacity – such as adjusting reactivation furnace conditions.

The two point measurement methods in Figure 5 provide better characterization of the adsorption performance of the activated carbon. The two point measures use controlled adsorption conditions to define an intermediate adsorption energy level and quantifying the volume of adsorption capacity above that energy threshold. Correctly chosen, the lower energy level reflects the total functional adsorption capacity for a given application and the upper energy level provides insight into a secondary consideration, such as adsorption of small organic compounds that impart taste and odor.

The most widely accepted two-point method is the Butane working capacity test, ASTM D5228. This method takes a dried sample of carbon at 25 Celsius and measures the weight gain upon being equilibrated in pure butane at one atmosphere, as depicted in Figure 4. The weight gain is termed “Butane Activity”. The sample is then purged with 1000 bed volumes of dry air over 40 minutes and the residual weight gain is measured and termed “Butane Retentivity”. The difference is termed the “Butane working capacity”.

De facto, the Butane Activity measures total adsorption capacity above a relatively low adsorption energy threshold and represents essentially the total pore volume. Butane Retentivity measures the capacity of those adsorption sites above an energy level that represents roughly one third to one half of virgin activated carbon capacity. While the total adsorption capacity measured by Butane Activity is worth knowing, a significant portion of this capacity is too low in adsorption energy to be of any practical value in an actual adsorption application.

The butane working capacity analytical method can be performed with any challenge gas, not just butane. Less easily adsorbed gases provide measures of higher adsorption energy thresholds. Two convenient alternatives to butane are propane and R134a (1,1,1,2 tetrafluoroethane, the HFC replacement for automobiles). These alternatives tend to measure activated carbon capacities with adsorption energies that are sufficient to be of utility in industrial applications.

One of the major requirements of any analytical technique used for process control is that it is easy-to-perform, affordable and fast enough to allow feedback to the processing conditions. In that sense, the methods of Figure 5 all have merit. If one forgives the practicalities of process control and cost per analysis, then one can look at analytical research techniques – which optimize the amount of information measured over the practicality of the assay.

Figure 6 (3) describes one such approach, which quantitatively measures available pore volumes over the entire distribution of adsorption energies and generates something called the “Characteristic Curve”. Unfortunately, the apparatus to generate such a “characteristic curve” is a research tool that is assembled from individual components and there is no standardized design. Some hapless researcher assembles an apparatus, runs countless calibration curves on known standards, then collects data on unknown carbons in hopes of gaining some insight. As such, this approach is of limited value in exchanging quantitative data. However, as a conceptual tool and for teasing out subtle differences between two carbons, characteristic curves really do tell the whole story.

Figure 7a shows the differential characteristic curve for Calgon CPG carbon, which is a benchmark carbon within the sugar industry. Also shown on Figure 7a are the corresponding thresholds for the butane and propane working capacity assays, as defined by Activity and Retentivity energy levels. Figure 7b shows the cumulative adsorption capacity for CPG carbon and represents the integral under the curve of Figure 7a, evaluated starting from the highest to lowest energy levels. Figure 7b is the experimental curve that is actually recorded by the experimental apparatus, with Figure 7a being generated by taking the derivative of the cumulative curve. Figure 7c is the cumulative adsorption curve plotted on a semi-log scale, which results in a smooth curve with uniform curvature. All three figures depict the same data, just treated differently mathematically to provide options for comparing curves from different carbons.

Since the log plot of the cumulative adsorption capacity tends to be the most uniform curve, it is usually used to compare different carbons. Such a comparison is shown in Figure 8 for many of the most popular commercial activated carbons. While it may be tempting to conclude that all carbons look the same, based on Figure 8, it should be noted that small differences on a semi-log plots often represent significant performance differences in the real world. In addition, the curve labeled “React”, signifying a high-temperature reactivated carbon, is discernibly below the other curves in the lower adsorption potential range, signifying lower total adsorption capacity.

In summary, the adsorption capacity of either virgin or regenerated activated carbon can be assayed to determine the available adsorption capacity. The measurement can consist of either one or two single point measurements, which quantify the total adsorption capacity above a specific adsorption energy threshold, or the entire “characteristic curve”, which maps the entire

distribution of adsorption capacity as a function of adsorption potential. The single point criteria are well suited for process control applications, while the entire adsorption distribution is still fundamentally a research tool.

The Effect of Activation on the Internal Structure of Virgin Activated Carbon

As noted previously, the dominant industrial practice for regeneration of spent activated carbon is high-temperature reactivation. This approach shares operating conditions and equipment with the process originally used to manufacture the virgin adsorbent. As such, there is merit to briefly reviewing how the activation conditions create activated carbon.

Figure 9 (3) summarizes the steps and reactions occurring during the activation process. The feed is the calcined raw material, meaning that the starting material has been heated above 800 Celsius in an oxygen-free environment. These conditions drive off all volatiles, including residual oxygen and hydrogen, and produce graphite plates that form the internal structure of the activated carbon particles.

The key difference between the calcining step and the activation step is the presence of an oxidizing gas. Figure 10 (3) shows the reactions of the three choices for oxidizing gases: water vapor, carbon dioxide and oxygen. The most popular choice is steam, which undergoes the “water gas” reaction with carbon to produce hydrogen and carbon monoxide. Oxygen would be less expensive, but the large reaction exotherm results in local heating of the graphite plates, which precludes accurate control of the reaction rate and does not produce uniform activation throughout the carbon particle.

On a molecular level, the oxidizing gas, steam or carbon dioxide, produces a gradual and uniform removal of the graphitic backbone as the activation process progresses. Figures 11a through 11d (3) depict the sequence by which the internal adsorption sites are modified by the activation process. Four main trends are occurring simultaneously as internal carbon is removed from the individual particle: the particle density is decreasing; the volume of adsorption sites is increasing; the energy of the individual adsorption sites is decreasing; and the yield of product, on a weight basis, is decreasing.

The characteristic curves are a fascinating way to capture the progression of the activation process, as shown in Figure 12 (3). The calcined starting material is the lower left curve with a density of 0.809. Upon activation, higher density activated carbon is formed with the highest amount of high energy adsorption sites (RHS) and the least amount of lower energy sites (LHS). As activation proceeds, the carbon density decreases, high energy sites are lost and a much larger number of low energy sites are gained.

Another way of envisioning this progression is shown in Figure 13 (3), where the effect of the activation process in an individual graphite plate is depicted. Since the graphite plate is the structural backbone of the carbon particle, there is a limit to the reduction of the plate width – which is the mechanical failure of the particle. This concern will reappear during the discussion of high-temperature reactivation that follows.

A Brief History Lesson

The development of the high temperature reactivation process is more of an addendum to the manufacture of activated carbon than an independent development. Figure 14 (3) shows the chronology of the domestic (as in United States) activated carbon industry. Coconut shell carbons were the earliest industrial activated carbons. WWI established the role of activated carbon in warfare and when the Japanese threatened the Allied supply of coconut-based material, a wartime research effort was undertaken to manufacture activated carbon from coal feedstocks. After WWII, the industrial capacity for the production of activated carbon from coal was free to pursue peacetime applications.

In a parallel development, the sugar refining industry was utilizing calcined bone char to remove both ash and color during sugar production. The combination of the sugar industry's desire to identify alternatives to bone char and the excess post-war capacity for activated carbon production resulted in the adaptation of activated carbon to sugar decolorization.

Since bone char was regenerable and reusable, this manufacturing practice was not inconceivable for the activated carbon alternative. Conveniently, the same equipment that activates carbon can be used to reactivate it and the processing conditions are essentially a subset of the conditions used to manufacture virgin material. Consequently, basically due to a combination of prior industry practice and coincidence, the process for the high temperature reactivation of spent activated carbon was "invented". This also explains why activated carbon manufacturers, who already own the equipment, are strong advocates of the high temperature reactivation technology.

The Effect of Reactivation on the Internal Structure of Spent Carbon

Lest one forget, the original charter of this paper was to evaluate the dominant regeneration technology, high-temperature reactivation, versus the alternative currently under development, low-temperature regeneration. With that in mind, the starting point is spent activated carbon, generated by adsorbing soluble compounds onto the internal surface of the activated carbon.

Figure 3c, previously discussed, depicts the internal graphite plates within a micropore region of coal-based activated carbon. Figure 15 (3) depicts the same region flooded with adsorbed compounds. The goal of the regeneration is to reverse the process of adsorption and return the carbon to the virgin structure, to the extent that is possible.

Unfortunately, there is no such thing as the "perfect regeneration" for applications like sugar decolorization. This conclusion is driven by the characteristics of the adsorbed color bodies, which range from sucrose size molecules to large molecular weight polysaccharides, as discussed by Godshall (5). Furthermore, these colorants are deposited within the activated carbon by diffusion from a liquid medium. Thus, when the spent carbon is dried out for regeneration, the colorants desiccate and bind more tightly to the internal surfaces of the carbon. In this form, the only option for removal is to decompose them in-situ by chemical reactions.

The conditions utilized for high-temperature reactivation are identical to those used for the original activation of the virgin carbon. However, the starting material is spent carbon, which differs from the starting material for activated carbon by the presence of the adsorbed color bodies. As the spent carbon is heated in the absence of oxygen, the effect on the graphitic backbone of the carbon is negligible, since the original manufacturing process encountered these conditions previously. The impact on the adsorbed color bodies is a different story – they dehydrate, devolatilize and form a residual material that is called “char”.

Figure 16 (3) summarizes the effects of pyrolysis and calcining conditions on spent carbon. The key conclusion is that char, which results from the thermal decomposition of the adsorbed color bodies, and the graphitic backbone of the activated carbon itself are chemically identical but physically very different. Figure 17 (3) summarizes what happens to the char under the gasification conditions, which is basically the same “water gas” reactions previously encountered during the activation process. As such, during reactivation, both the char and the graphitic backbone of the carbon are gasified.

The sequence of adsorption, followed by devolatilization to form a residue in the carbon pores, calcination to convert the residue to char, and reactivation to create reactivated carbon is worth exploring, especially when repeated multiple times. Figure 18a (3) outlines the conditions used for the series of figures that follow (Figures 18b through 18l (3)). The simulation uses an adsorbate, phenol, that forms a relatively high portion of char and the carbon is heavily loaded each cycle to accelerate the effects of the reactivation cycle. It is expected that sugar decolorization carbon would not exhibit as rapid a transformation, although the overall features of the metamorphosis would be expected to be the same as demonstrated with phenol.

Figure 18b shows the starting virgin activated carbon, followed by Figure 18c with the first cycle of adsorption to form spent virgin carbon. Figure 18d shows the reduction of the adsorbed phenol into residue and Figure 18e shows the reactivated carbon, where most of the char and a portion of the graphitic backbone of the original carbon have been removed. This process continues until Figure 18k, which depicts the reactivated carbon after five adsorption and reactivation cycles.

The cumulative effect of the multiple reactiverations can be seen by comparing Figure 18b, showing the virgin carbon internal structure, with Figure 18k, the internal structure after five reactivation cycles. While the reactivated material continues to posses adsorption capacity, as depicted in Figure 18l, the energy levels of the adsorption sites have decreased. Figure 19a (3) shows the estimated adsorption sites and corresponding energy levels for the virgin carbon and Figure 19b (3) provides the same exercise for the five fold reactivated material. The effect of the multiple reactiverations is to create new low-energy sites by the removal of graphitic backbone and to plug high-energy sites with char.

The progressive conversion of adsorption capacity into accumulating char and monotonic erosion of the graphitic backbone of the carbon particle does have an ending, but not a happy one. Figure 20 (3) shows the fate of the particle, which is a shattering of the particle into fines when the graphite plate width reaches some minimal size. Comparing Figure 20 with Figure 13, it is apparent that the effect of reactivation on plate width is virtually identical to the effect of additional activation. The difference is that the reactivation process also deposits char in the

internal pores of the carbon, reducing useable adsorption capacity. One way of envisioning 0.55 AD (apparent density) carbon after five high-temperature reactivations is superimpose 0.22 grams per cubic centimeter of accumulated char over a virgin carbon backbone of 0.33 AD.

Introduction to Low-Temperature Regeneration Alternative for Spent Carbon

The phenomenon of low temperature oxidation of activated carbon in a source of molecular oxygen, typically air, is not new. A body of work performed at the University of Alicante (6) in Spain about 25 years ago explored the evolution of previously activated virgin carbon in air at 350 Celsius. As noted in the Discussion section of the first paper:

At high percentages burn-off and as a consequence of the weight loss, the adsorptive properties of the active carbons is diminished but they can still be considered good active carbons even when the weight loss is about 50%. This could be important from the industrial point of view, since these carbons could stand temperatures up to 350°C for long periods of time (several days) with no other inconvenience than their reduction in weight.

Additional papers (7) further characterized the adsorption characteristics of the activated carbons as air at 350 Celsius was used to modify the internal adsorption sites over a range of up to 70 percent weight loss or “burn-off”. After these four papers, the researchers seemed to drop this line of inquiry and additional work by this group is not reported in the literature.

The only potential industrial application of the air oxidation of activated carbon appears to be Dussert et al. (US 5,368,738) (8), where a method of conditioning virgin or reactivated carbon by air oxidation at 300 to 700 Celsius for between 5 minutes and three hours is claimed to control initial pH and alkalinity in water treatment applications. The potential popularity of this approach is seriously undermined by the very next patent issued, Dussert et al. (US 5,368,739) (9), where the same benefit can be obtained by wetting the carbon and allowing it to air oxidize at ambient temperatures for longer periods of time.

What is new about the oxidation of activated carbon by air is the effect on other oxidizable organics present within the activated carbon. As summarized in the abstract for a recent paper on the topic (10):

Abstract: CarbOxLT (which stands for Carbon Oxidation at Low Temperatures and pronounced Car-Box-Lite) is a recently discovered phenomenon whereby activated carbon exhibits a slow controllable exothermic oxidation in air over a temperature range of 190 to 300 degrees Celsius (374 to 572 degrees Fahrenheit). CarbOxLT also refers to a wide class of useful applications for activated carbon under conditions that feature the slow oxidation regime. Those applications include the oxidation of vapor phase organics, the destruction of organic liquids and the regeneration of spent activated carbon. The paper will first cover the studies of the core CarbOxLT phenomenon involving the controlled oxidation of activated carbon in air. Subsequently, the applications and considerations for applying the technology to the oxidation of organic vapors, organic liquids and the regeneration of spent activated carbon will be discussed. Finally, a review of the current understanding and engineering implications of the CarbOxLT phenomenon will be presented.

Further applications of the technology are discussed in a recent U.S. Patent Application (11) and associated PCT filing. In the patent application the use of low-temperature regeneration technology for regenerating spent activated carbons from sugar refining is demonstrated (and subsequently claimed).

With such a limited amount of formal study on the low-temperature regeneration phenomenon, it is premature to state precisely what occurs at the molecular level. A future paper is anticipated (12) to review additional piloting results to date and summarize the current understanding of the phenomenon. The succinct hypothesis is that the chemisorbed oxygen that predominates on the surface of activated carbon over the temperature range of the low-temperature regeneration, as discussed in Lear et al.(13), reacts with the adsorbed organics present in the spent activated carbon. The overall reaction mechanism appears similar to the mechanism proposed by Wu et al. (14) for the platinum-catalyzed oxidation of BTX on activated carbon, except that the oxygenated surface of the activated carbon replaces the role of the oxidized platinum catalytic sites.

While the underlying science of low-temperature regeneration may take some time to sort out, the application to the regeneration of spent sugar decolorizing carbons is straightforward to demonstrate. Since considerable time has been devoted to exploring the effects of high-temperature reactivation on spent activated carbon, that technology will be used as the benchmark for comparison.

Tracking the Course of a CarbOxLT Regeneration

While the background rate of oxidation of the activated carbon has been measured as slow to very slow, as discussed in Reference 6, the rate of oxidation of spent activated carbon under similar conditions is significantly more rapid. A sample of spent virgin activated carbon, heavily loaded with sugar refining color bodies, was regenerated over a total of about 24 hours at progressively higher temperatures. The results of the propane working capacity assay, performed at each temperature plateau, are shown in Figure 21.

As can be seen in Figure 21, at each progressively higher regeneration temperature, incremental recovery of adsorption capacity is measured (Note: the sum of Propane Retentivity and Working Capacity equals the Propane Activity). Figure 22 shows the variation of density at the progressively higher temperatures, in addition to the propane assay results (the "Dried in N₂" is plotted at 50 Celsius for convenience, the actual sample was dried at 150 Celsius in dry nitrogen). As would be expected, as the regeneration proceeds and adsorbed material is removed, the bulk density of the activated carbon media decreases.

Figure 23 shows the trends of the propane assay as a function of density. The plot shows the direction of the regeneration from left to right, as indicated by decreasing density. The properties of the virgin carbon are shown as the far right data points, at density = 0.471. It is apparent that at progressively higher regeneration temperatures, the properties of the original virgin carbon are incrementally approached. It is equally apparent, especially in the propane retentivity trend, that the virgin activated carbon properties will not be attained when the regenerated carbon reaches

the virgin carbon density of 0.471 grams/cm³. As such, low temperature regeneration is not a “perfect” regeneration, in the sense of recreating virgin carbon properties.

The trends of Figure 23 indicate that the low temperature regeneration recovers most but not all of virgin carbon properties, a conclusion that has been observed in repeated laboratory regenerations of spent carbons loaded with non-volatile adsorbates. Two further comparisons are in order: how does low temperature regeneration compare with the high temperature reactivation of the same spent carbon and how does the deviation from virgin carbon properties evolve over multiple adsorption-regeneration cycles.

Figure 24 shows a comparison of high temperature reactivation and low temperature regeneration, performed on the same batch of spent carbon used for Figures 21 to 23. Figure 24 includes the properties of dried spent carbon and spent carbon that has been calcined (heated to 850 Celsius in a dry nitrogen atmosphere – which reduces any adsorbed compounds to char). Virgin carbon values for propane activity, propane retentivity and density are used to normalize the data shown in Figure 24, hence all virgin carbon properties are shown as 100%.

As can be seen in Figure 24, the dried spent carbon shows a significant increase in density and loss of propane capacity compared to the virgin carbon. Calcining the spent carbon recovers the majority of the propane capacity loss, but also converts the remaining adsorbed material into non-volatile char, which would accumulate over repeated adsorption-calcining cycles.

Calcining followed by steaming constitutes the high temperature reactivation process, since the steam removes the char by the water gas reaction. This allows the density of the reactivated carbon to be returned to near virgin carbon density, yet the propane capacities are still significantly decreased from the original virgin carbon levels. Basically, high temperature reactivation results in some loss of carbon skeleton and leaves some residual char, with the sum of the two opposing effects resulting in virgin carbon density. Unfortunately, char has very poor adsorption properties compared to the activated carbon skeleton and that tradeoff is reflected in the decreased propane adsorption capacities.

Finally, the results for the low temperature regeneration are shown on the far right of Figure 24. As discussed in conjunction with Figure 23, the regenerated carbon approaches but does not attain virgin carbon properties. The data shown in Figure 24 is for a different regeneration of the same spent carbon as Figure 23 and the second regeneration results were slightly better than before. However, compared to the starting virgin activated carbon, the propane capacity decrease for the low temperature regeneration is roughly one half that experienced with high temperature reactivation.

Comparing the Performance of Regeneration Options Over Multiple Cycles

As discussed, the actual industrial practice is to regenerate and recycle carbon adsorbents to “extinction”. As such, the cumulative effect of multiple loading and regeneration cycles is important. A laboratory study was performed to simulate the evolution of “pool” carbon properties.

The comparison starts with virgin activated carbon (Norit GAC1240, another typical sugar decolorizing carbon) and simulates the adsorption step by equilibrating the carbon with a controlled ratio of industrial molasses. The industrial molasses, supplied by a domestic refiner of cane sugar, was obtained after multiple strikes on the affination stream generated during the initial washing of incoming raw cane sugar. During the equilibration step, the color bodies from the molasses adsorb into the activated carbon, which is then “sweetened-off” with water rinses and regenerated. The sequence is repeated several times, without the addition of any make-up virgin carbon, in order to establish the trend over multiple adsorption-regeneration cycles.

It should be noted that the equilibration step required only a few hours and, as such, was significantly more rapid than the typical operating cycle of actual sugar decolorizing adsorbers. In addition, the adsorption was accomplished from a solution containing much higher concentrations of color bodies and much lower levels of sucrose and other simple sugars. As such, it is likely that the adsorption phenomena are similar and representative, but not identical, to the adsorption phenomena encountered in actual sugar decolorizing applications.

As a starting point, Figure 25 shows the trend of multiple adsorption-regeneration cycles for virgin GAC versus activated carbon that is regenerated by calcining. The regeneration of spent carbon by calcining alone is not a recommended industrial practice because the adsorbed organics are reduced to char. Decomposing the adsorbed organics to char does recover some adsorption capacity, but the resulting char accumulates in the regenerated activated carbon and progressively consumes adsorption capacity. However, calcining alone does represent a reasonable “worst case” regeneration, just as restoring the spent carbon to virgin activated carbon properties represents a reasonable “best case”.

As shown in Figure 25, the calcining alone of spent carbon results in a progressive loss of adsorption capacity, as measured by the uptake of propane defined by the “Propane Activity” assay discussed previously. In addition, calcining alone results in the stepwise increase in the measured density of the regenerated carbon, further evidence of the accumulation of char inside the carbon.

High-temperature reactivation is basically just calcining followed by the “water gas” reaction. The addition of steam reacts with the char (and graphitic backbone of the carbon) and returns the density of the carbon to near virgin levels. In this manner, the monotonic accumulation of char is avoided, but the structural integrity and operational life of the carbon particle is shortened, as previously discussed.

Figure 26 shows the trend over multiple cycles for high-temperature reactivation, in addition to the previous trends of Figure 25. As can be seen, the steep loss of propane activity previously seen with calcining alone is avoided, although the capacity of the high-temperature reactivated carbon still declines a significant amount with each adsorption-reactivation cycle.

Figure 27 adds the trend for low-temperature regeneration to the previous trend of Figure 26. Low-temperature regeneration results in a noticeably slower rate of adsorption capacity loss, as measured by propane activity, than high-temperature reactivation. However, the loss of capacity compared to virgin activated carbon is still discernable. As such, low-temperature regeneration is

not a “perfect regeneration”, but rather a distinguishable improvement over the current industrial practice of high-temperature reactivation.

Figure 28 shows the same comparison for the three regeneration techniques using propane retentivity as the measure of the condition of the regenerated carbons. The overall trends are similar to the trends demonstrated by the propane activity assay, but the differences between the various regeneration methods is considerably less pronounced.

While it cannot be demonstrated from the data presented, it is anticipated that low-temperature regeneration conditions should result in significantly less attack on the graphitic backbone of the activated carbon as compared to high-temperature reactivation. The milder conditions should reduce the generation of fines during carbon transport, which is attributed to the mechanical weakening of the carbon particles. As such, for a circulating pool of activated carbon, the make up rate with virgin activated carbon is anticipated to be less for low-temperature regeneration than would be required for a similar system using high-temperature reactivation.

Summarizing the Differences Between CarbOxLT = LTR vs. Reactivation = HTR

In the previous section, in order to put the effectiveness of low-temperature regeneration in perspective with high-temperature reactivation, virgin activated carbon was used as the end point for the “perfect regeneration” and calcining alone was used as the other extreme, the “worst case” regeneration. Based on the measurement of propane activity as a quantification of the useable adsorption capacity of the regenerated carbons, the low-temperature regeneration demonstrated approximately half the adsorptive capacity loss per adsorption-regeneration as compared to high-temperature reactivation.

As discussed at the beginning of the paper, there is a strong interaction between the adsorption capacity loss per cycle, the make up rate of added virgin activated carbon per regeneration and the steady-state adsorption capacity of the regenerated pool carbon. For a given make up rate, the lower the capacity loss per cycle, the higher the pool capacity. For the case where capacity loss is decreased and the make up rate decreases, the pool capacity may improve or may decrease, depending on the magnitude of each change.

For those rare cases where the make up rate is so low that the pool capacity is depressed due to the cumulative capacity loss of the recycled carbon, it is generally possible to adjust the regeneration conditions to facilitate the loss of recycled carbon and thereby increase the make up rate. For high-temperature reactivation, additional steam will increase the make up rate. For low-temperature regeneration, operating the regeneration at a higher temperature will increase the make up rate.

It is not possible is to adjust regeneration conditions to decrease the make up rate without severely impacting the capacity loss per cycle. This occurs when insufficient steam is used for high-temperature reactivation and too low a regeneration temperature is used during low-temperature regeneration. Thus, there are minimum processing conditions for achieving an adequate regeneration that has an associated intrinsic make up rate, beyond which the make up rate can be artificially increased without significant deterioration of the capacity loss per cycle.

There are really no options for decreasing the make up rate below the intrinsic make up rate for an adequate regeneration without severely impacting on the extent of regeneration and associated capacity loss per cycle.

One can easily appreciate how the make up rate impacts on the cost of operating a carbon regeneration system, since the cost of purchasing the make up virgin carbon quantifies the cost. However, make up carbon costs rarely dominate the overall cost of operation of a carbon system, except in the case of “use once and discard” processes, such as with powdered activated carbon. There are other costs that are less obvious, which range from direct fuel costs for the regeneration equipment to depreciation of the capital investment of the regeneration capability.

Calgon published an article (15) that does a good job delineating the economics of operating a high-temperature reactivation system. Unfortunately, since the article dates from 1978, the utility and capital costs are not accurate for today’s industrial climate. However, the overall structure of the economic analysis is sound and comprehensive.

Relative to the high-temperature reactivation technology, the low-temperature regeneration approach has two significant advantages that impact on the economics of operation. These advantages are discussed in greater detail in Reference 12. The first advantage relates to direct operating costs, specifically the fuel requirements. As discussed in the Calgon paper (15), the fuel costs for high-temperature reactivation are several thousand Btu per pound of carbon processed. If an afterburner is required to destroy the volatiles generated during the charring of the adsorbates, the fuel requirement may well double. The high fuel costs are a direct result of operating at high temperatures and using the “water gas” reaction, which is endothermic – even at 850 Celsius (note: the “water gas” reaction is an entropy-driven reaction, which is why it does not occur at temperatures below 800 Celsius).

In contrast, low-temperature regeneration is exothermic and can use the heat generated by the oxidation of the adsorbed organics to supply the bulk of the necessary heat to raise the reacting mass to the desired regeneration temperature. The direct fuel costs for the low-temperature regeneration process are estimated at less than one-quarter the fuel costs for high-temperature reactivation.

A more significant advantage of the low-temperature regeneration process relates to the operating temperatures and the acceptable materials of construction. High-temperature reactivation requires refractory lined equipment and exotic alloys due to the high operating temperatures, 800 to 1000 Celsius. Low-temperature regeneration operates at temperatures of less than 400 Celsius, which allows for carbon and stainless steels without linings to be used for equipment fabrication. Not only are the initial construction capital costs significantly reduced, but the operational flexibility and reliability is greatly improved over refractory-lined designs.

The major differences between CarbOxLT (LTR) and Reactivation (HTR) are summarized in Figure 29. While both processes allow the recycling of spent activated carbon adsorbent, they are two entirely different approaches to the same application. The actual specifics of a low-temperature regeneration operation will not be known until one or more units are constructed and operated for a sufficient period of time. However, it is clear that the low-temperature

regeneration process enjoys several pivotal technical advantages over the high-temperature reactivation technology currently utilized by sugar refining operations worldwide.

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Figure 1.

Reactivation Process

Physical Carbon Losses during CCC type reactivation:

Overall losses can be 5% per cycle typical, 10% extreme:

1 to 5% can be due to gasification or O₂ in furnace

Less than 1% due to Handling in Herreschoff Furnace

Other Losses are the fines removed in:

Furnace stack/screening/back-washing

Fines generated in: Slurry feed/transport system,
dewatering screw,
truck/shipping
customer application
vessel loading/unloading.

Figure 2.

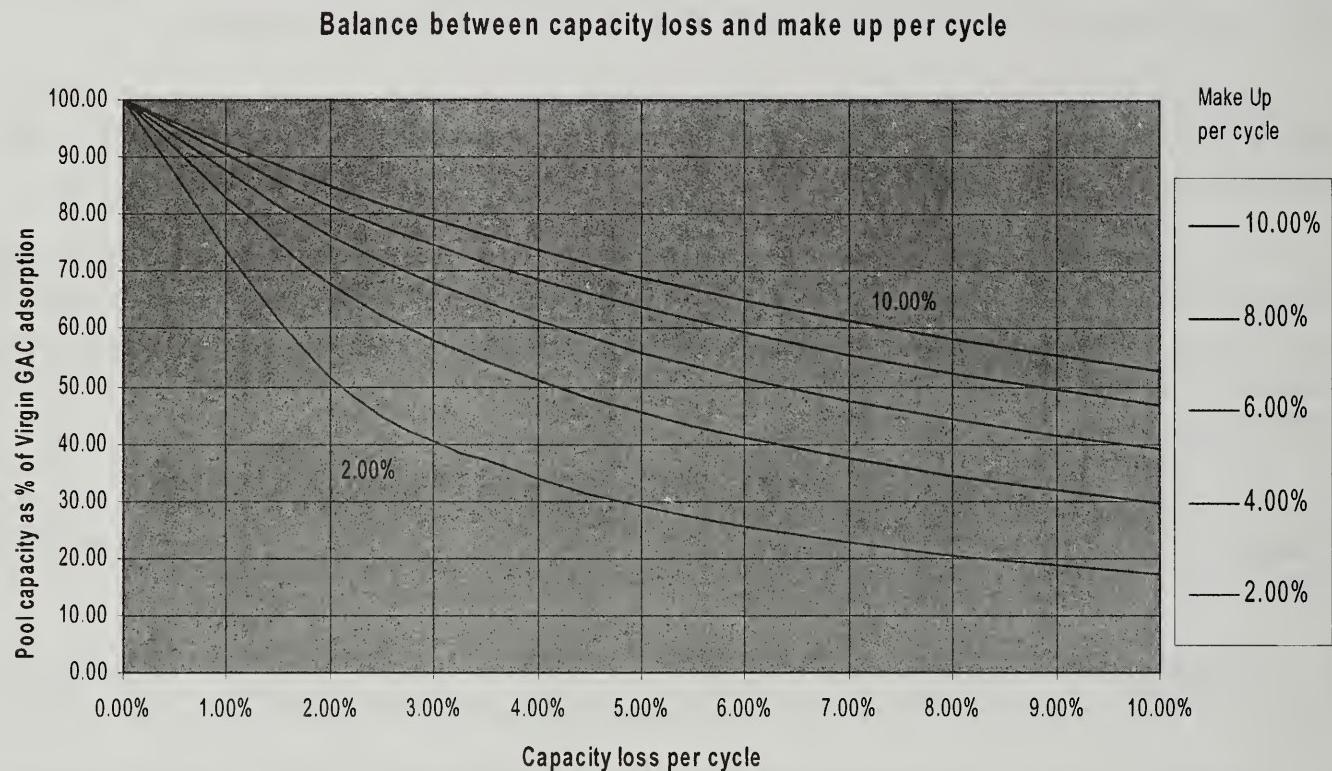


Figure 3a:

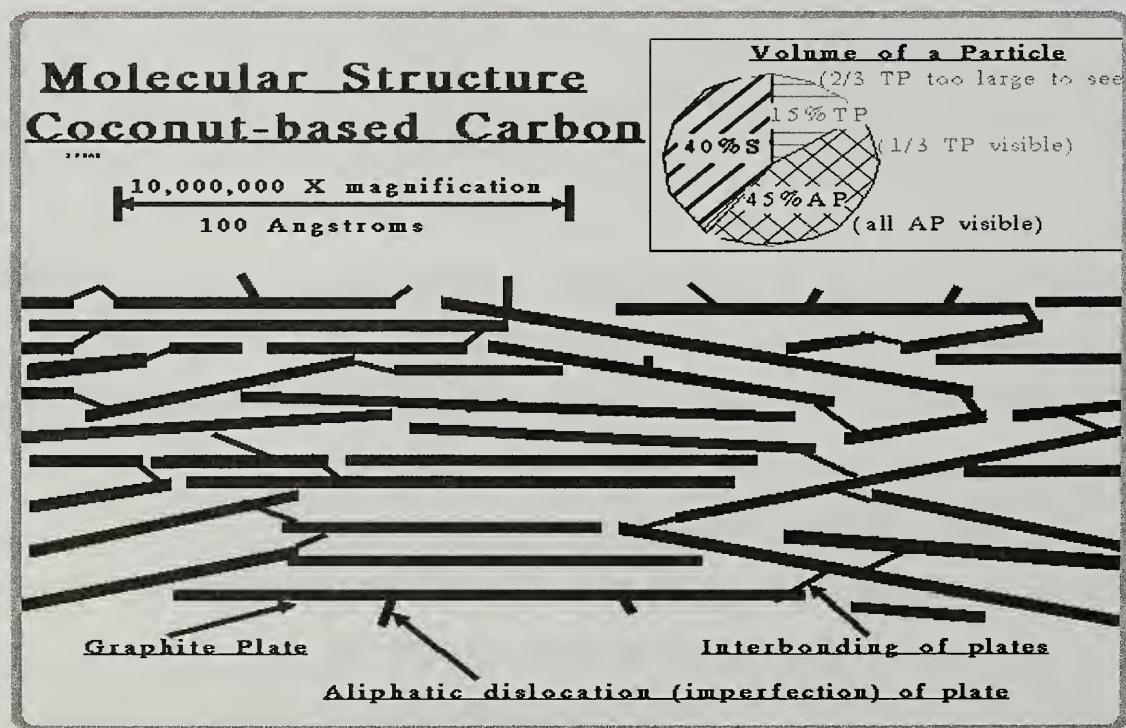


Figure 3b.

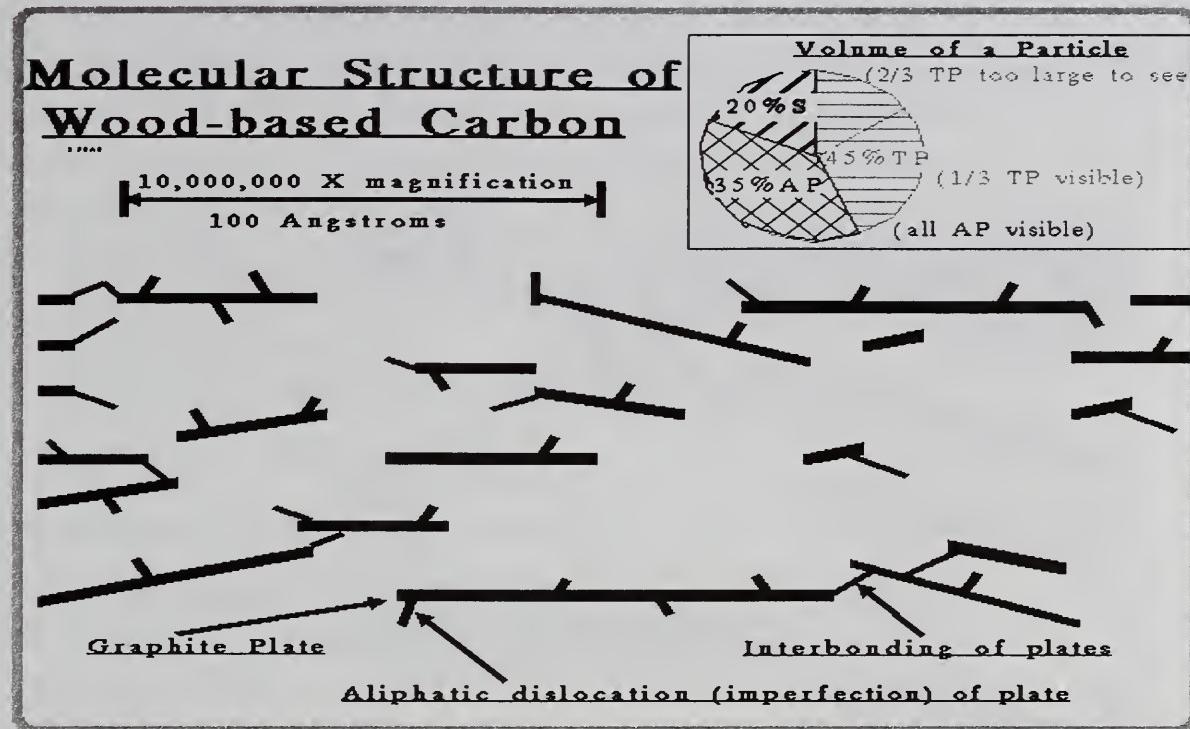


Figure 3c:

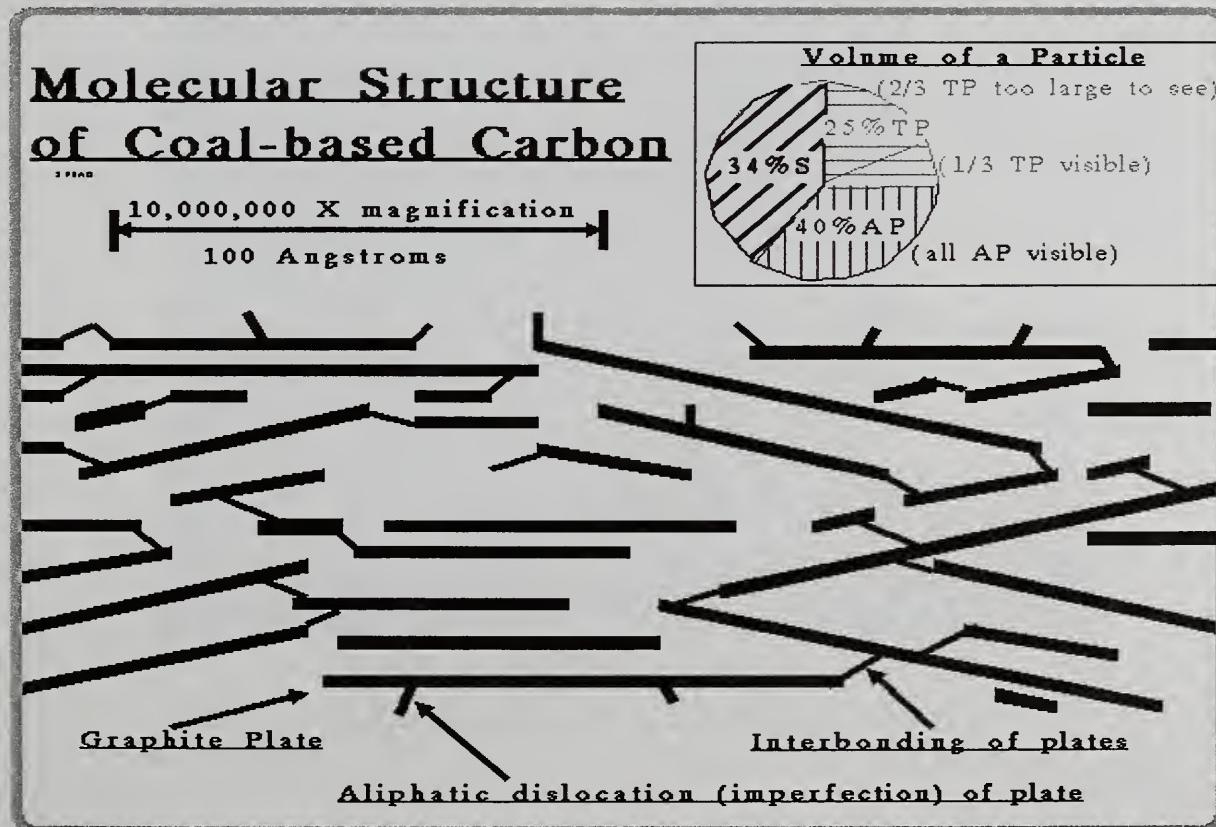


Figure 4.

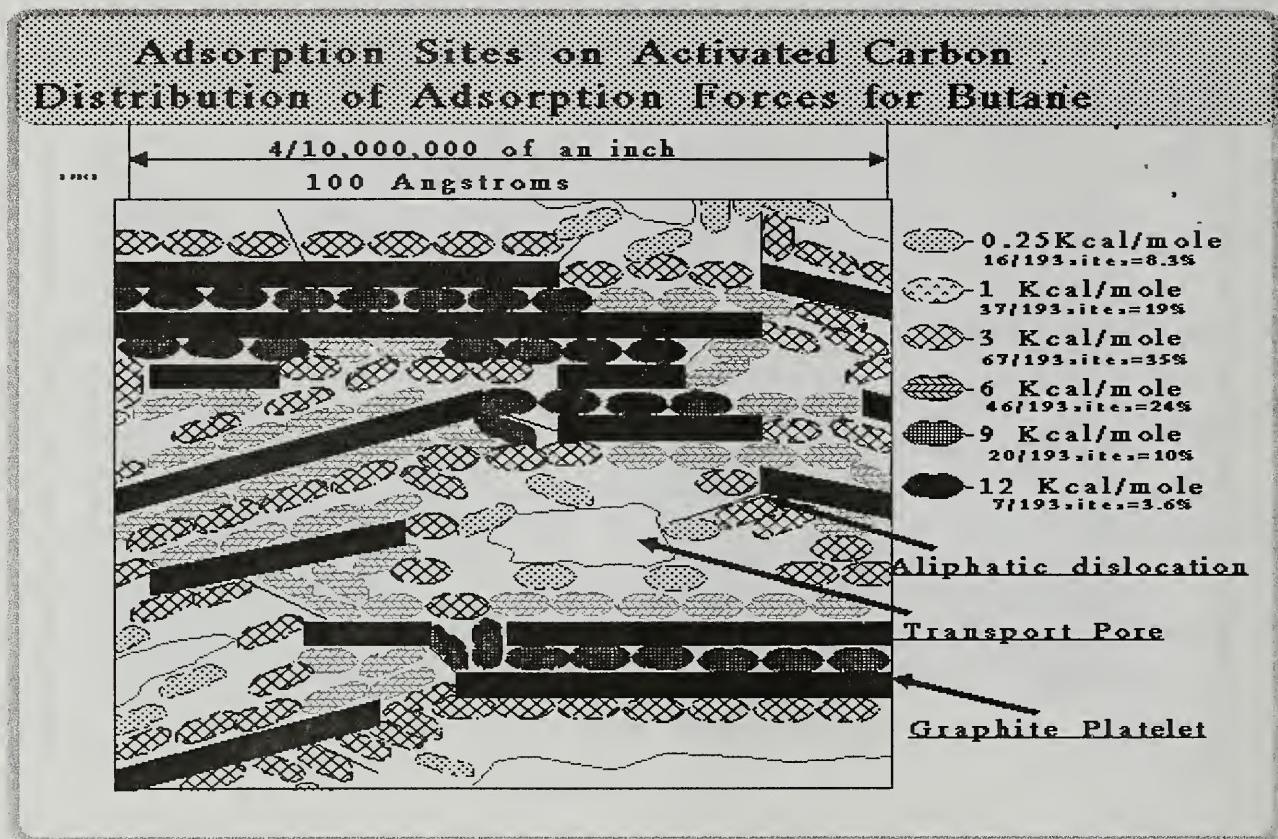


Figure 5.

How Carbon is Specified Now

- Single Point measures
 - Total adsorption pore volume
 - BET (Nitrogen Adsorption) surface area
 - Iodine number (ASTM D4607-90)
 - Carbon Tetrachloride number (ASTM D3467-88)
 - CFC-114 Activity (ASTM D5020-89)
 - Butane number = Butane Activity
 - Molasses Decolorizing Number
- Two point measures
 - Butane working capacity (ASTM D5228-92)
 - Activity – Retentivity = Working Capacity
 - R134a working capacity (ASTM D5228 w/ R134a)
 - Propane working capacity (ASTM D5228 w/ Propane)

Figure 6.

Characteristic Curves

The goal of a full characterization of activated carbon is the direct measure of this distribution of adsorption sites.

The adsorption potential, or adsorption energy, of the site is actually what is measured but is closely related to the adsorption force.

The number of sites is multiplied by the volume of the adsorbate molecules and the distribution is reported as pore volume versus adsorption potential, called a characteristic curve.

Figure 7a.

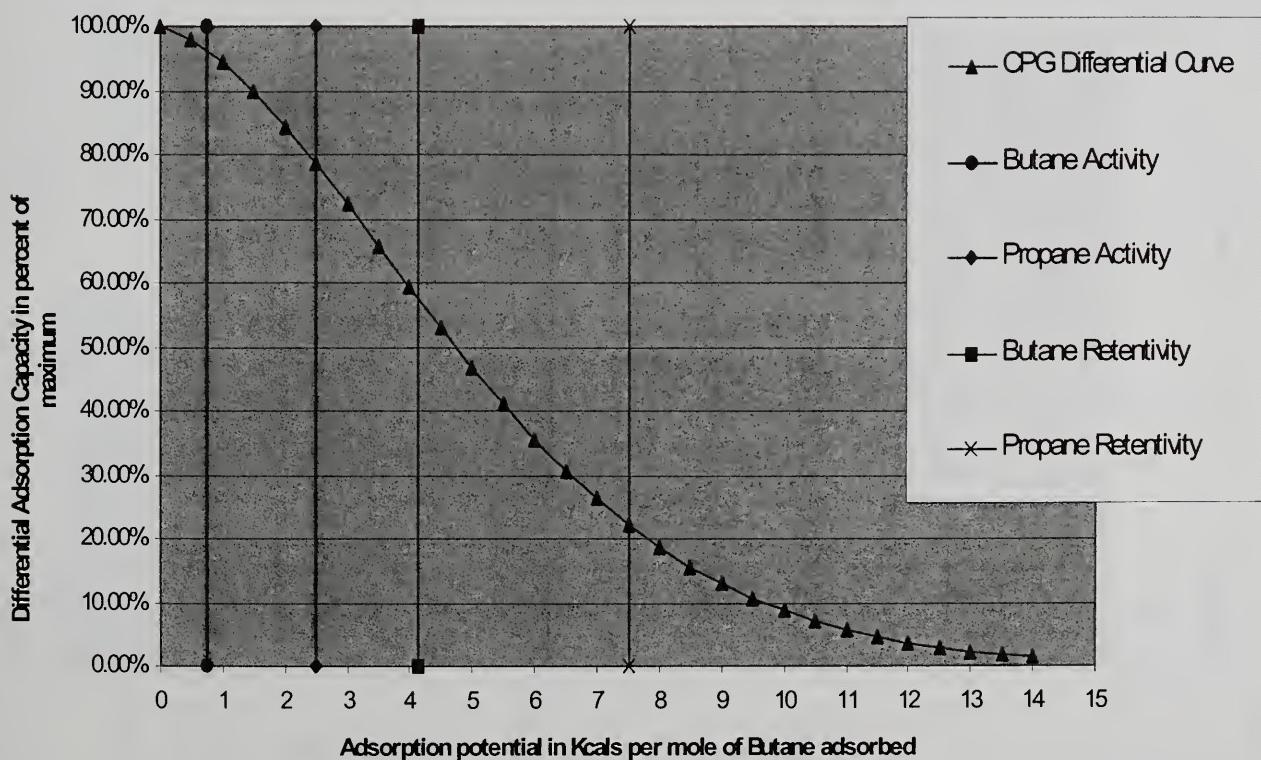


Figure 7b.

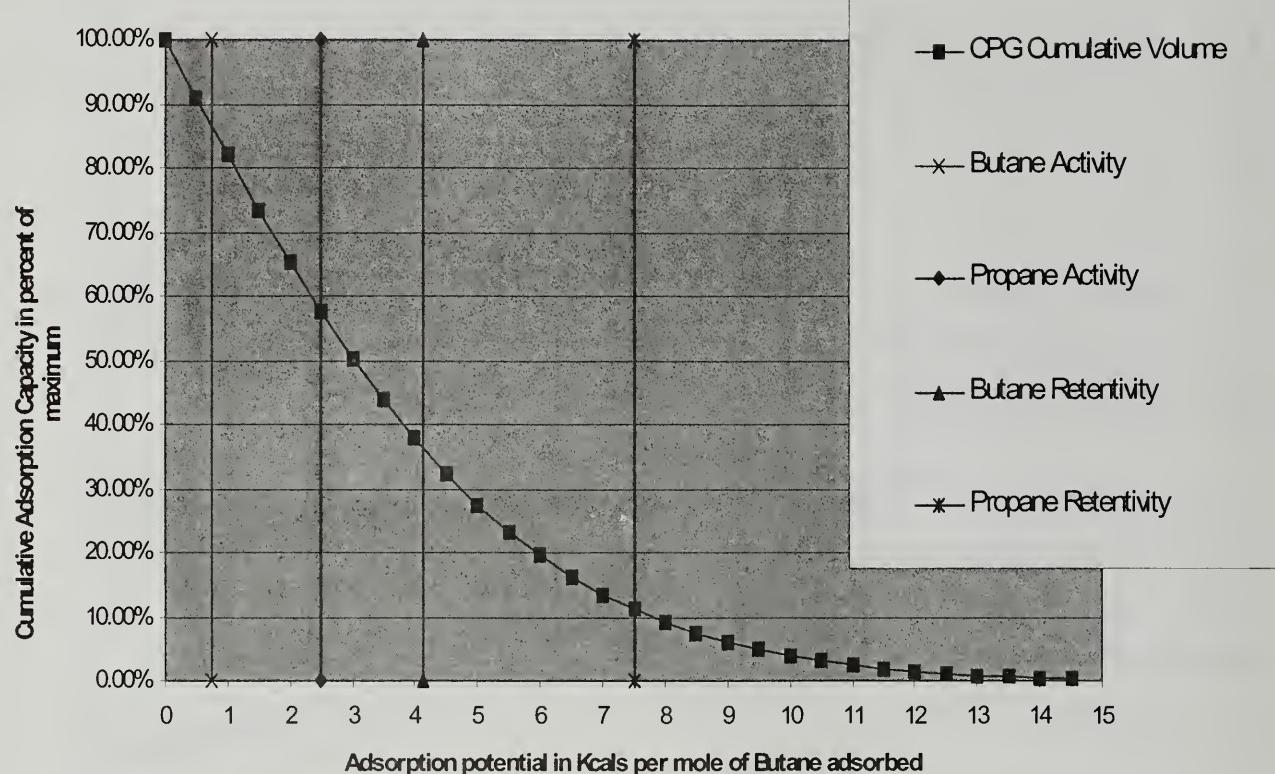


Figure 7c.

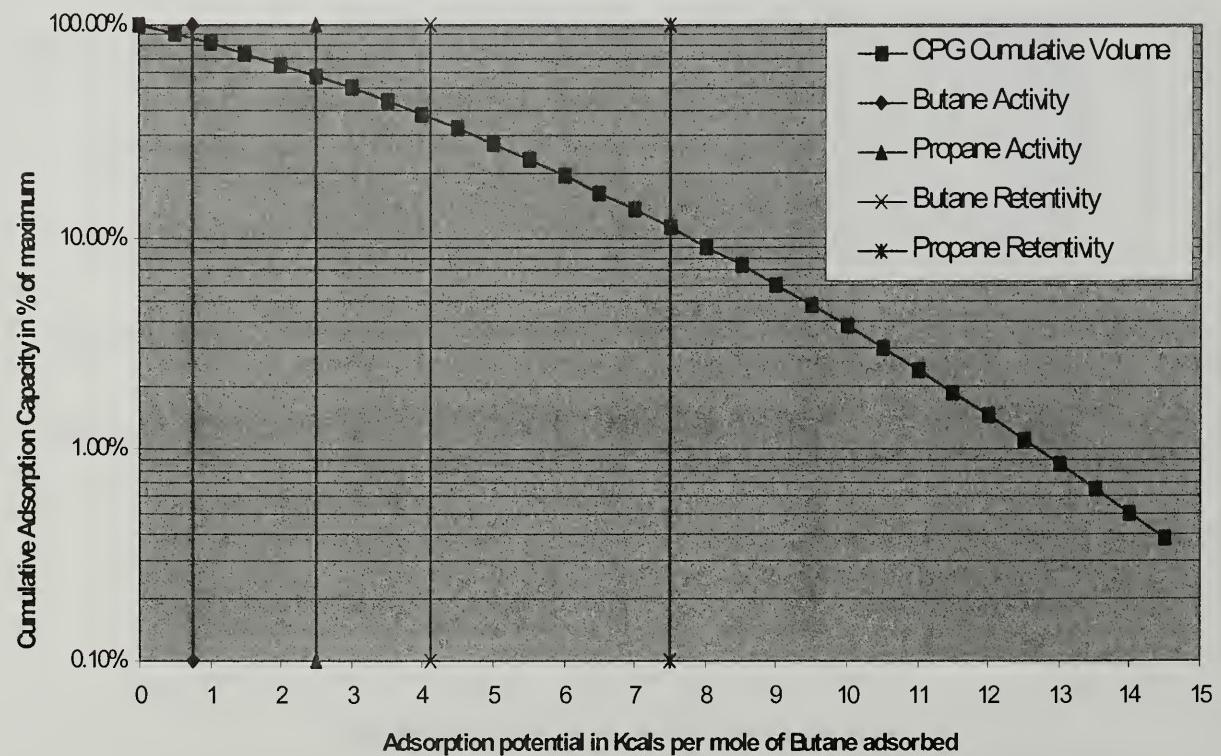


Figure 8.

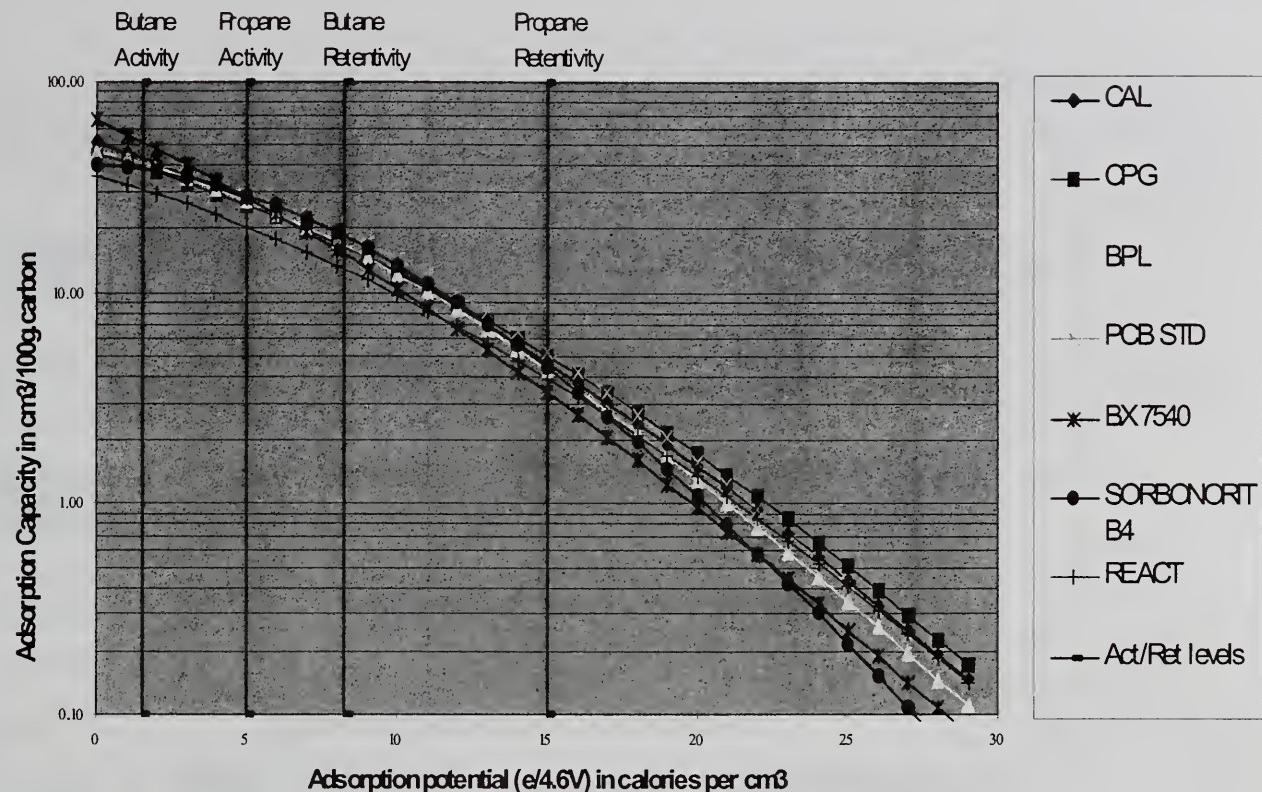


Figure 9.

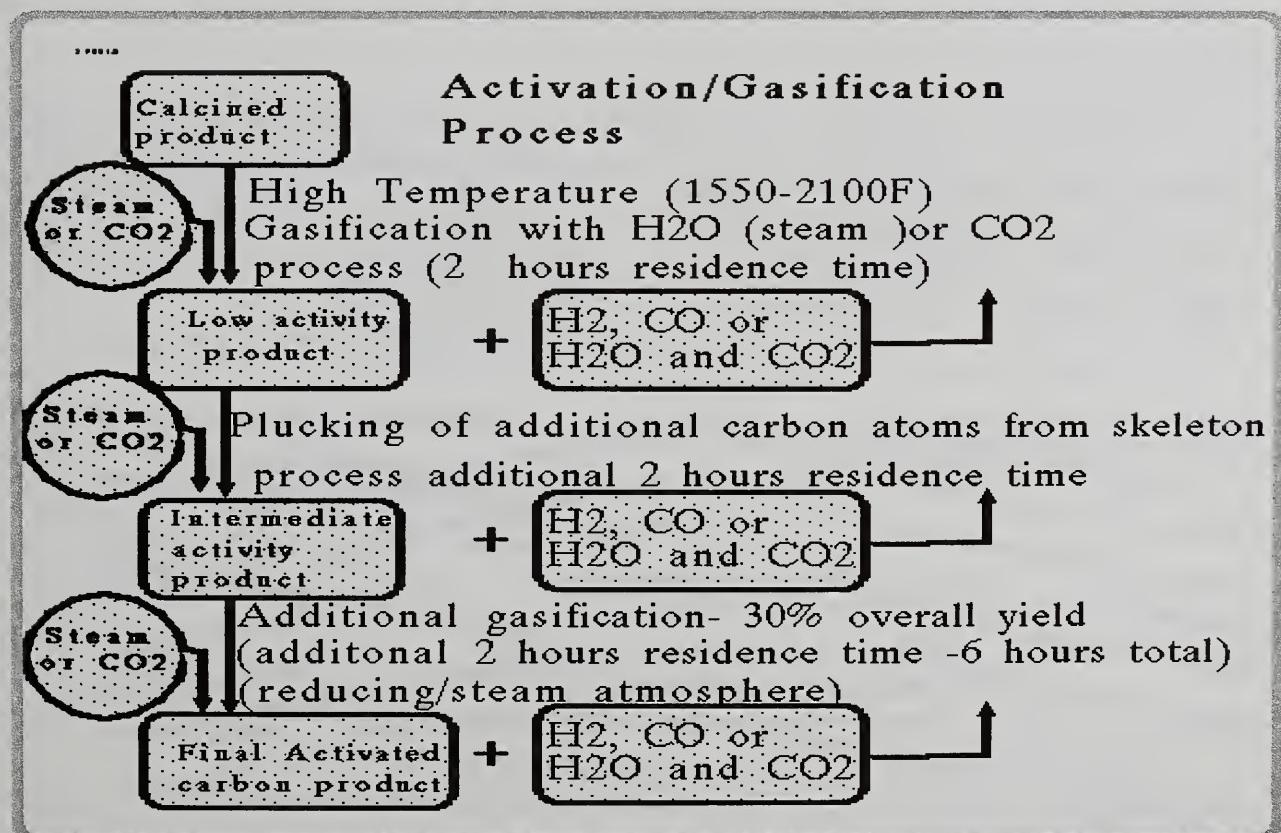


Figure 10.

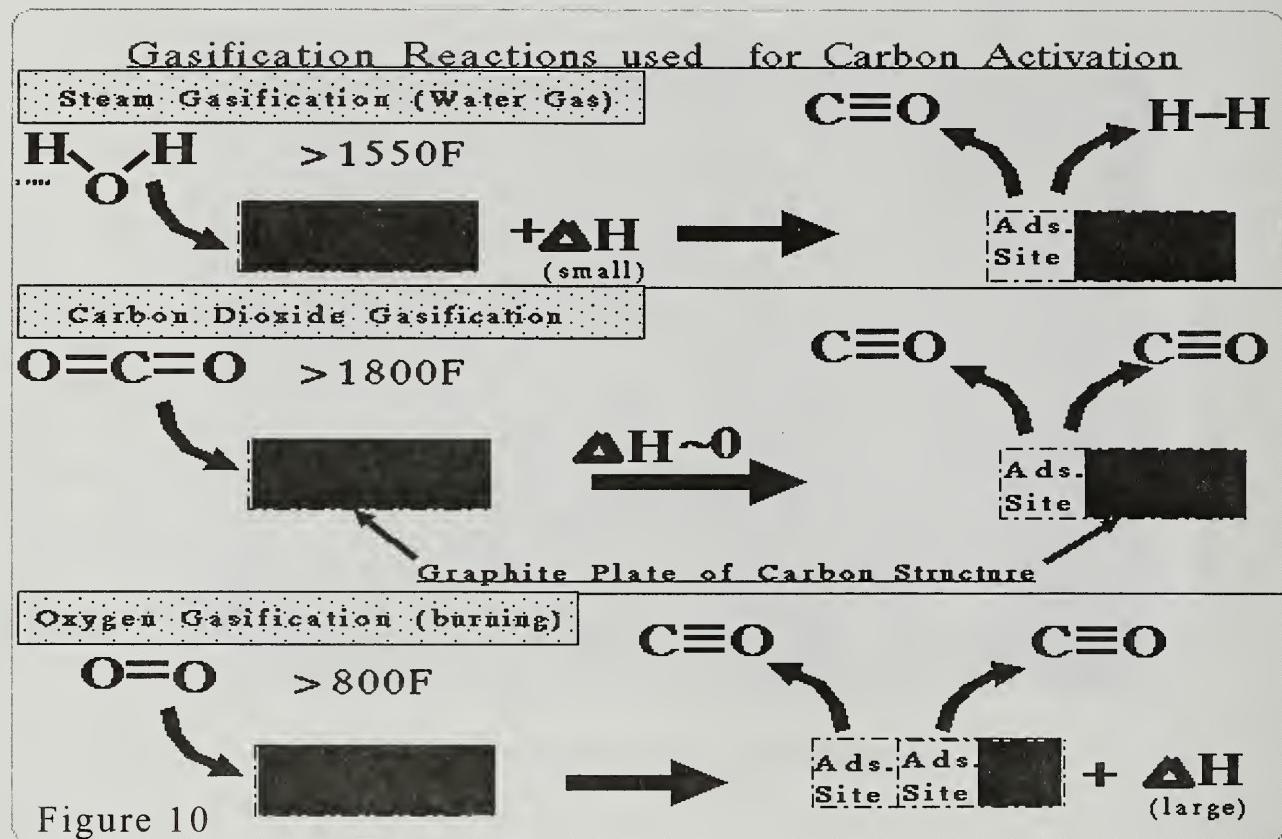


Figure 11a:

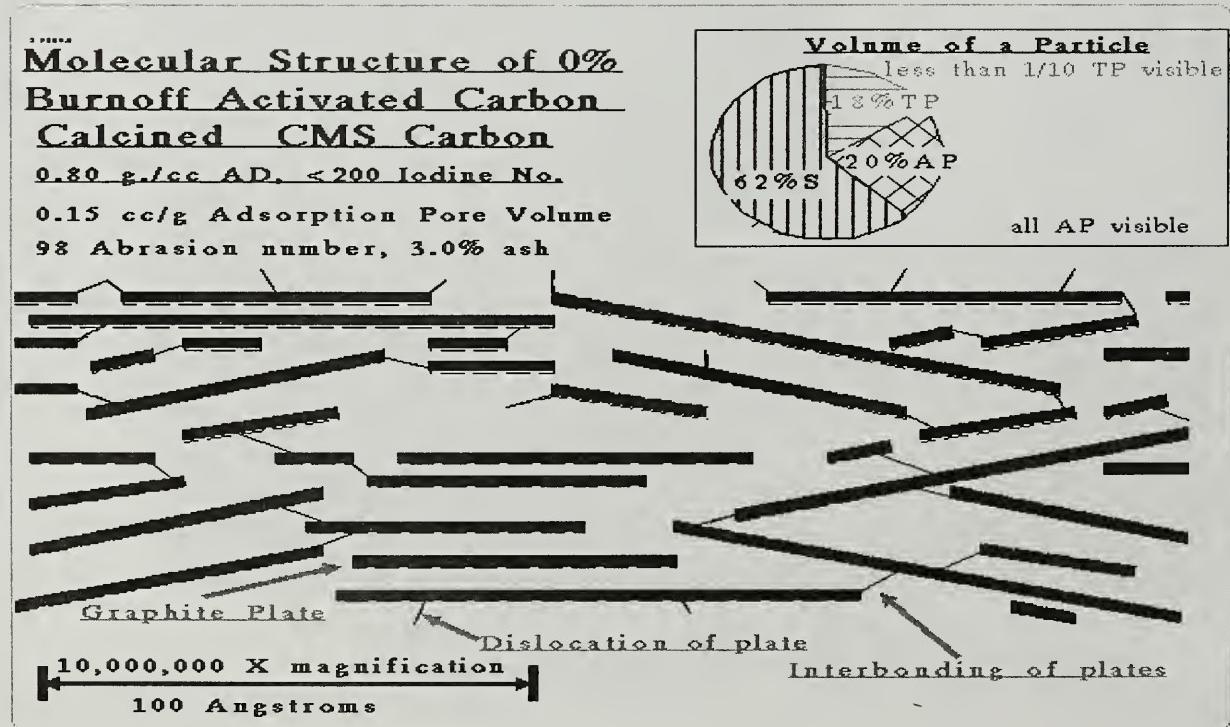


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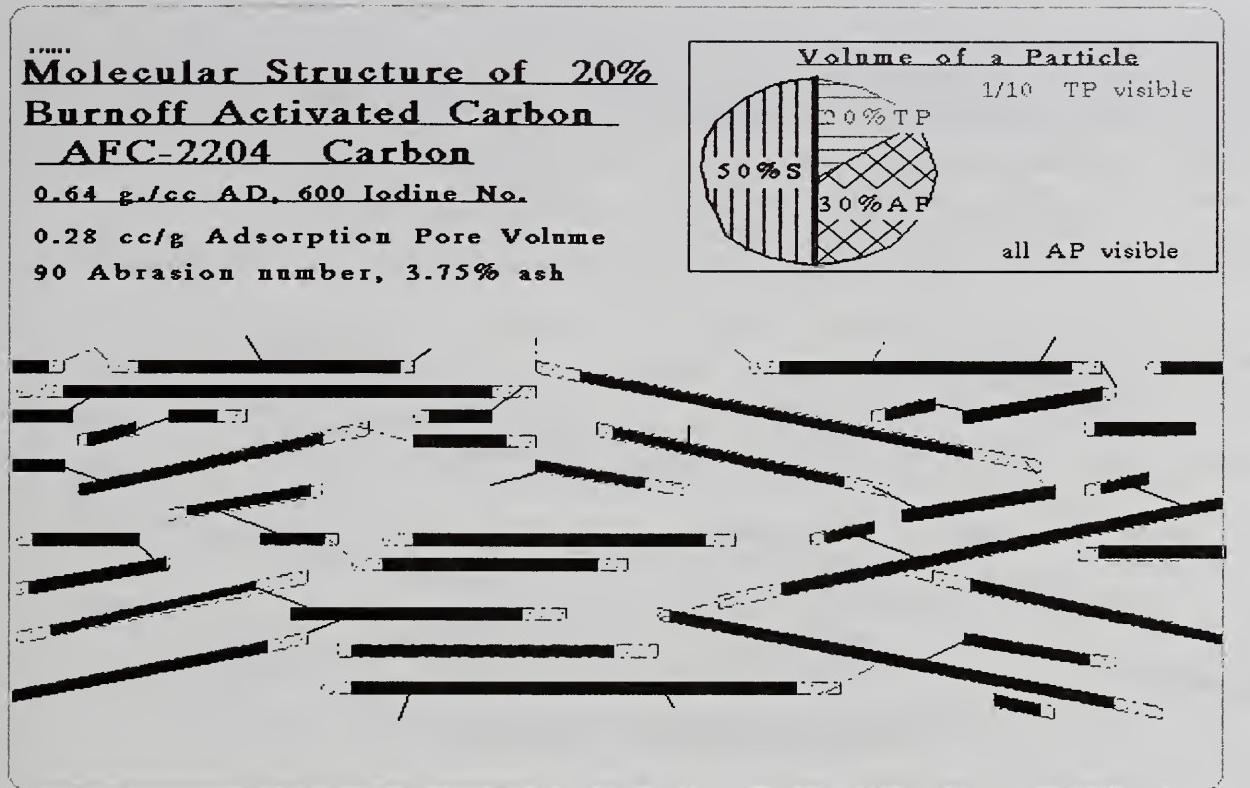


Figure 11c.

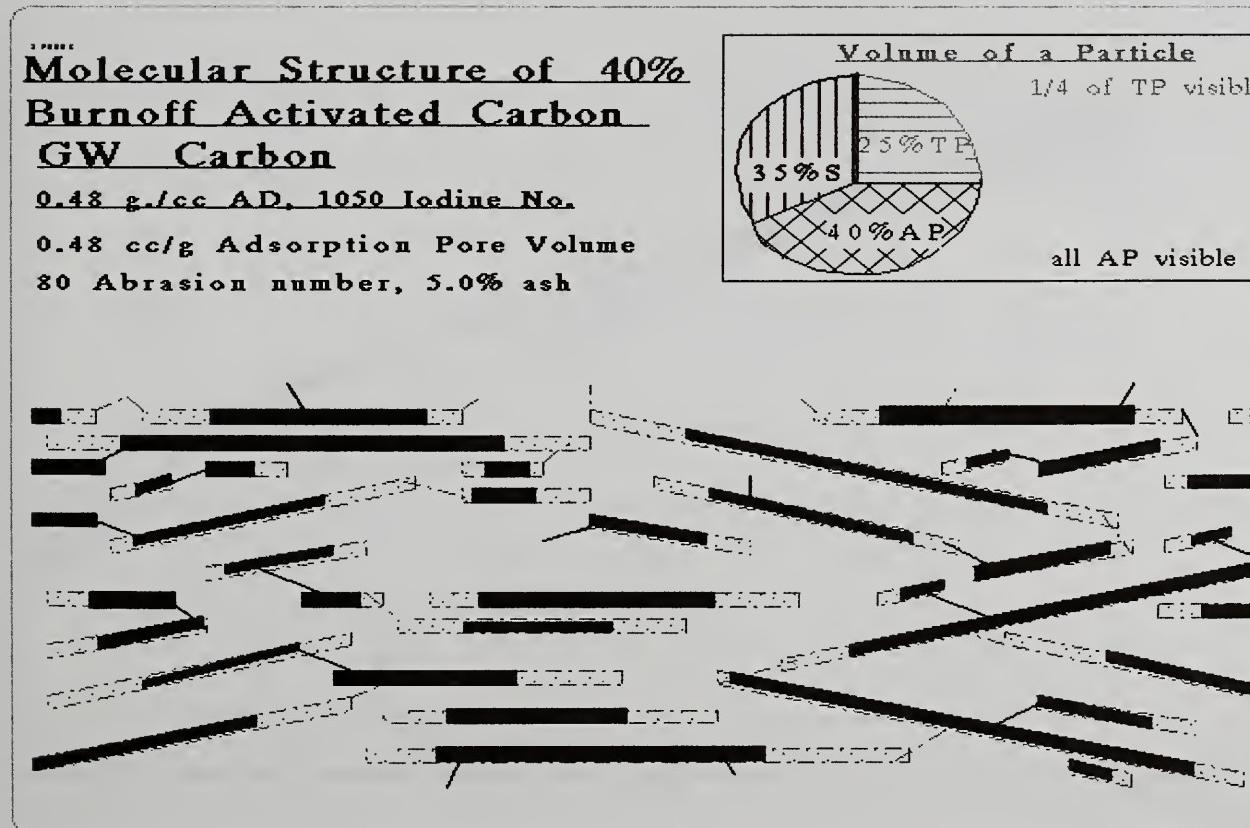


Figure 11d.

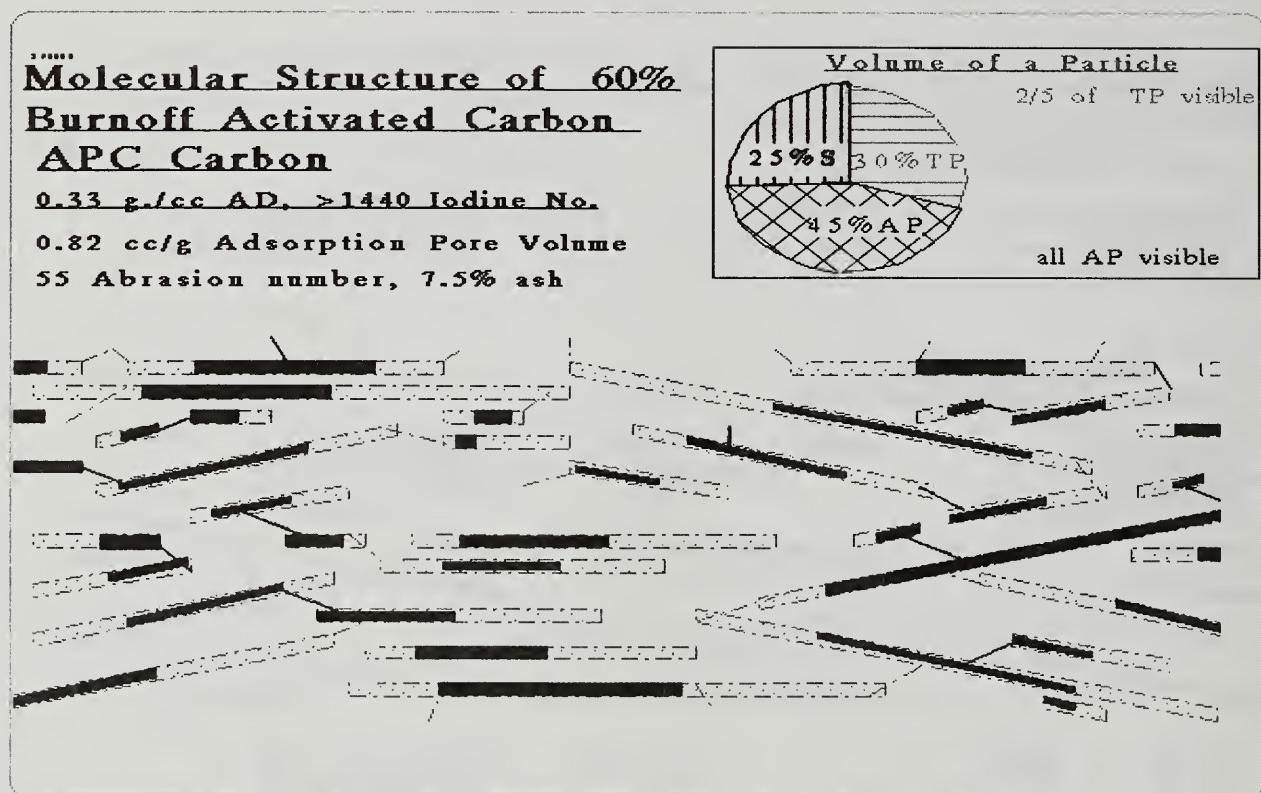


Figure 12.

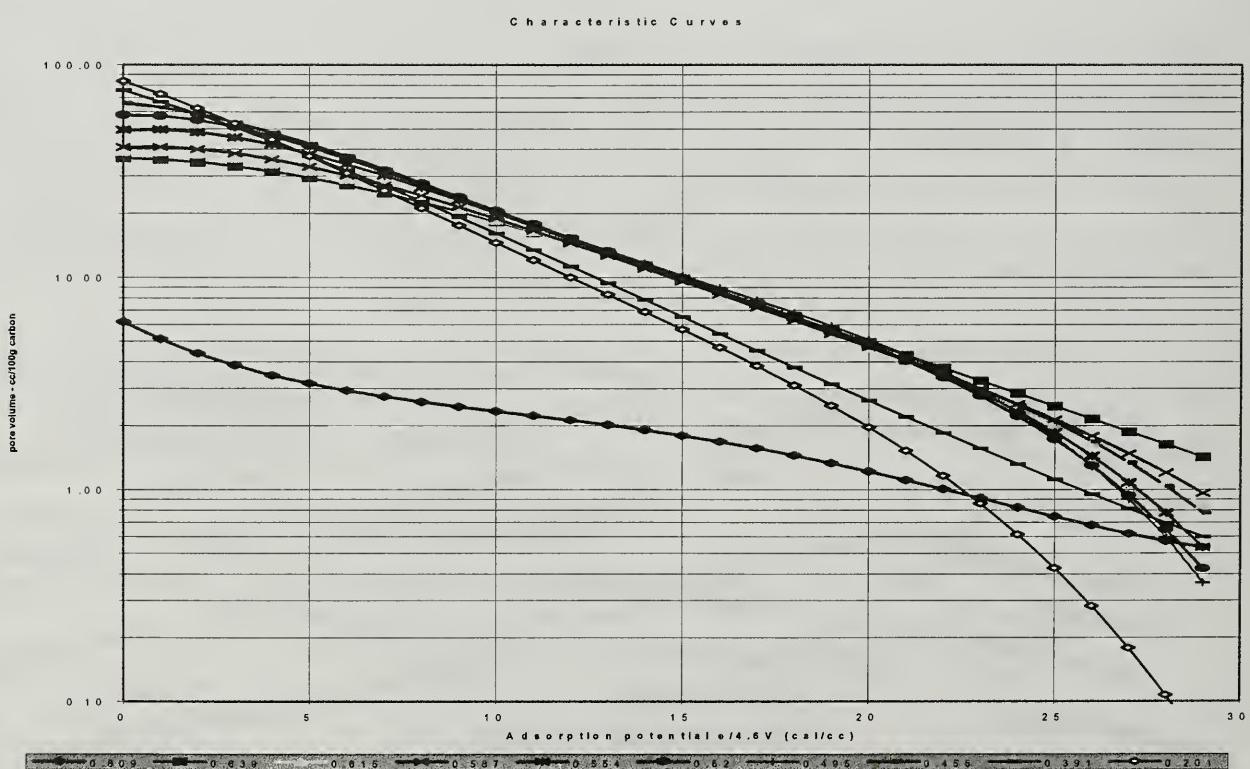


Figure 13.

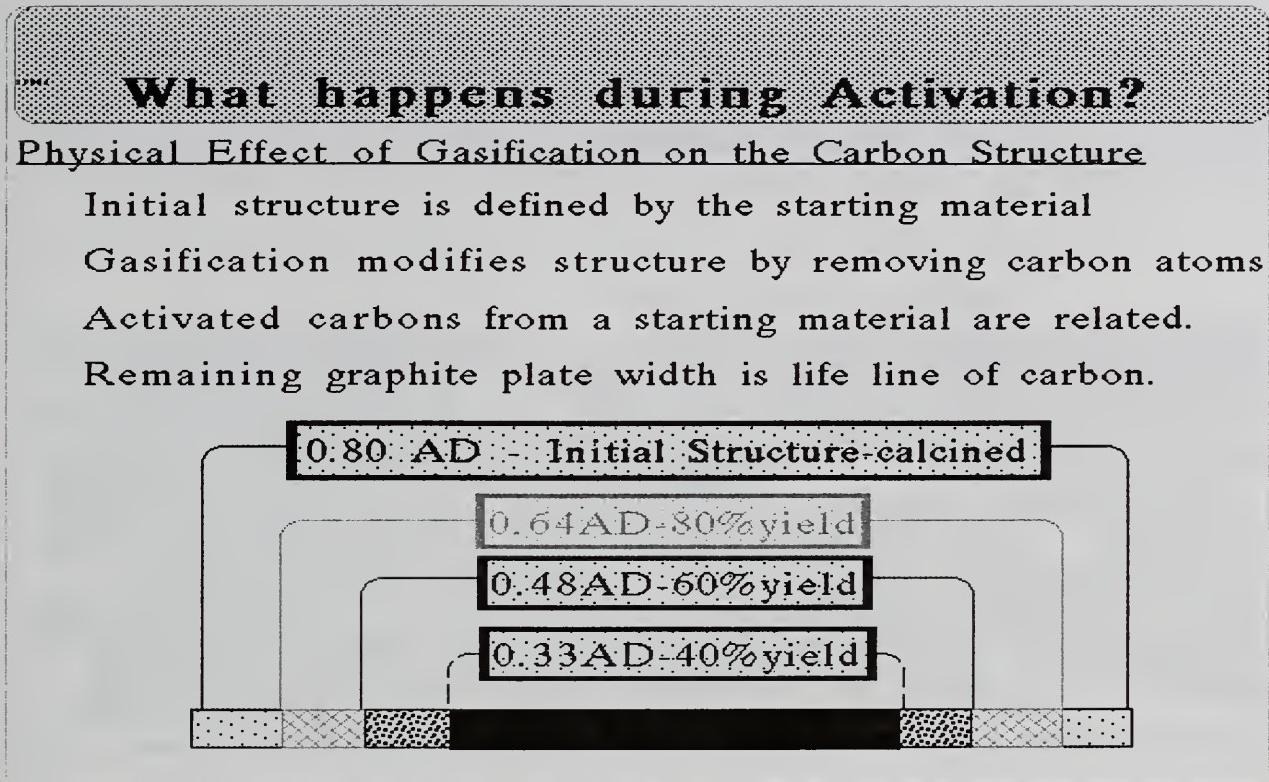


Figure 14.

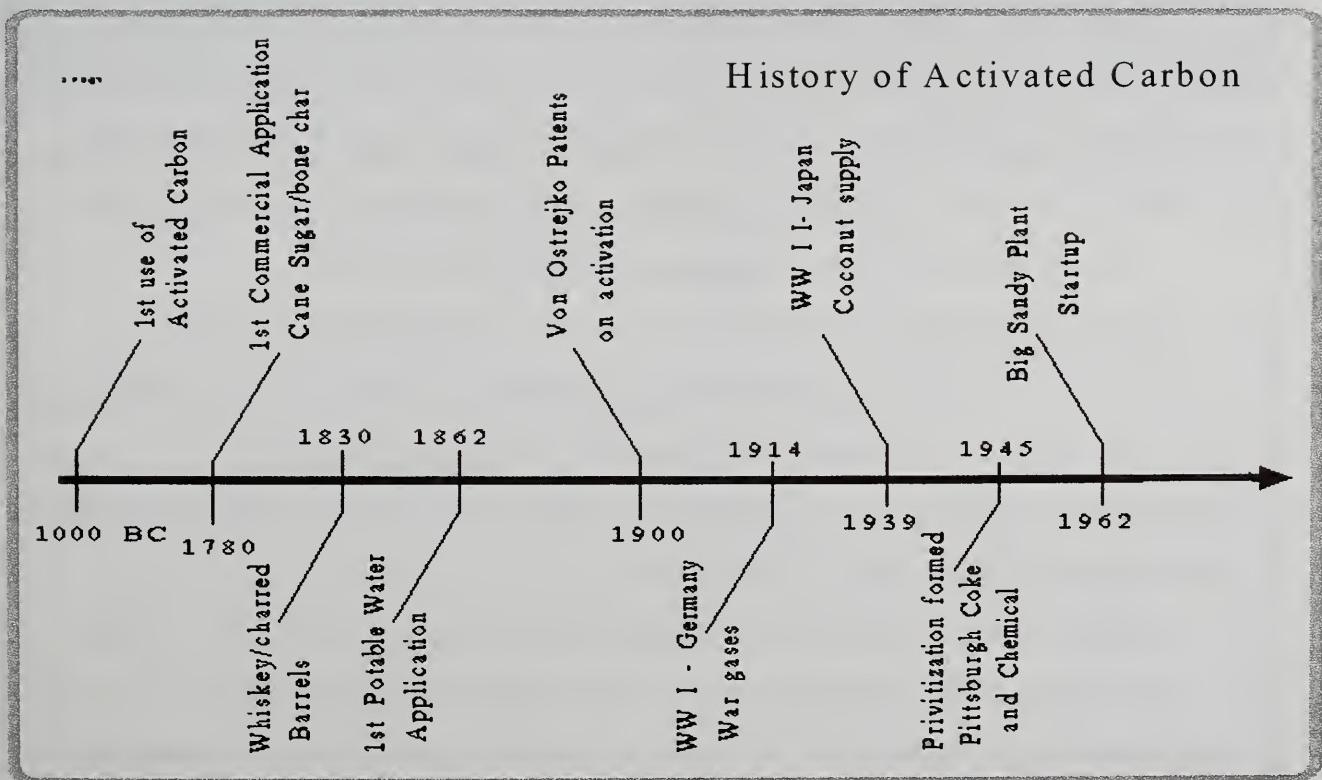


Figure 15.

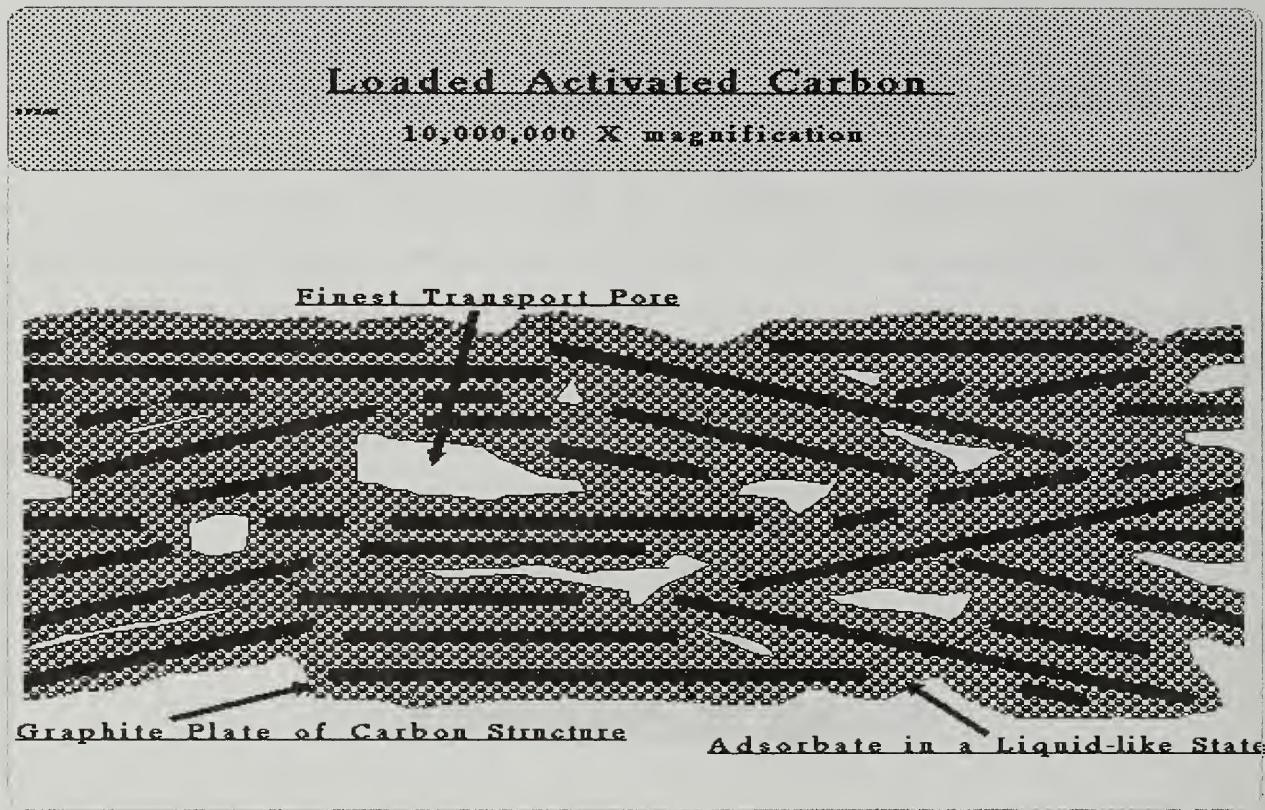


Figure 16.

Pyrolyzation of Spent Carbon

Pyrolyzation Process: Thermal Destruction of Adsorbates

- What doesn't desorb during devolatilization forms char in the adsorbing regions of the structure
- Char formation means a loss of adsorption volume
- Char is further condensed through thermal calcination to form a purer and denser form of carbon

• Amount and form of char is characteristic of the adsorbate

Adsorbate Char characteristics:

- Carbon may be partially graphitic (inert/low reactivity)
- Char is rarely porous - no adsorption properties
- Generally equal to or more reactive than carbon skeleton

Figure 17.

Gasification Reactions for Pyrolyzed Spent Carbon

Chemical nature and chemical reactivity of the Char

- Pyrolyzed and Calcined Char is Chemically similar to the activated carbon structure and graphite.
- Reactivity to activating gases can vary between chars
- Char reactivity is a function of the adsorbates on the spent carbon and drying/devolatilization/pyrolyzation processes
- Ratio of the reactivity (gasification rate at 1800F steam) of the char deposits compared to the carbon skeleton.
- Generally ratio less than unity (unity is worse case)

Figure 18a.

Effect of Reactivation Cycles on Activated Carbon

Only region of activated carbon structure affected by the adsorption/reactivation cyclic process is:

the molecular structure of carbon

(100 angstrom scale - 10,000,000 magnification)

Parameters for the reactivation simulation:

Virgin activated carbon: CCC Filtrasorb - 0.55 g/cc AD

Spent Carbon Parameters: 34 weight % loading of Phenol leaves 50 wt% char residue with a reactivity ratio of 1.0
91% overall carbon yield with each reactivation cycle

Reactivation specification: react till original 0.55 g/cc AD
1800F, 100% steam atmosphere, laboratory react furnace

Figure 18b.

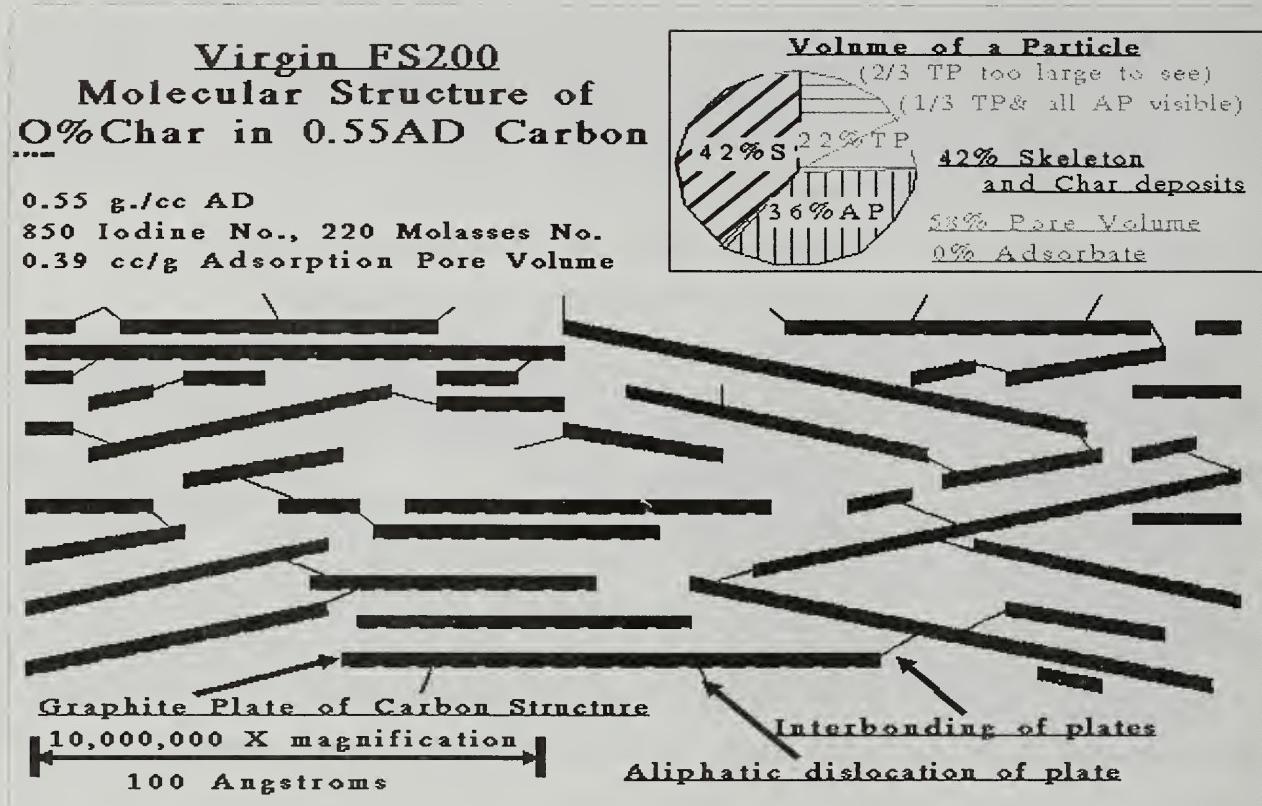


Figure 18c.

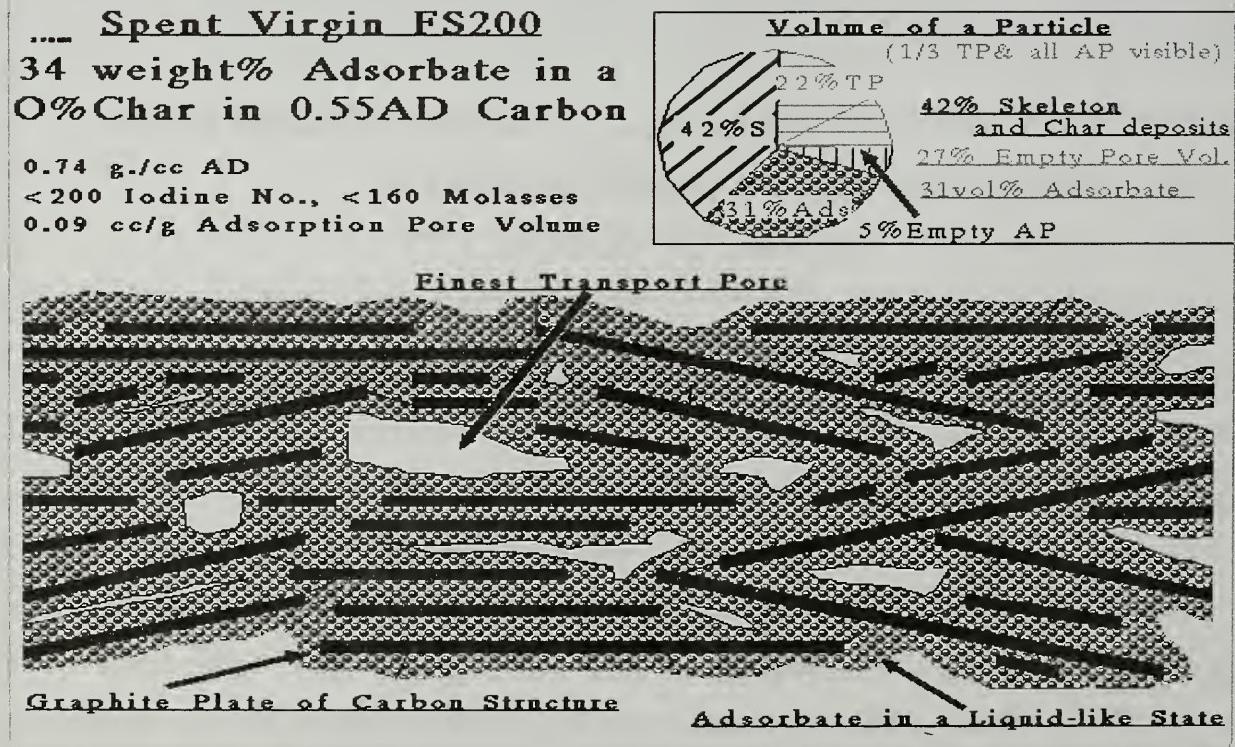


Figure 18d.

Devolatilized Virgin FS200
 18 wt % Residue in a
 0.55AD Carbon & 0% Char

0.65 g./cc AD

590 Iodine No., 200 Molasses No.

0.27 cc/g Adsorption Pore Volume

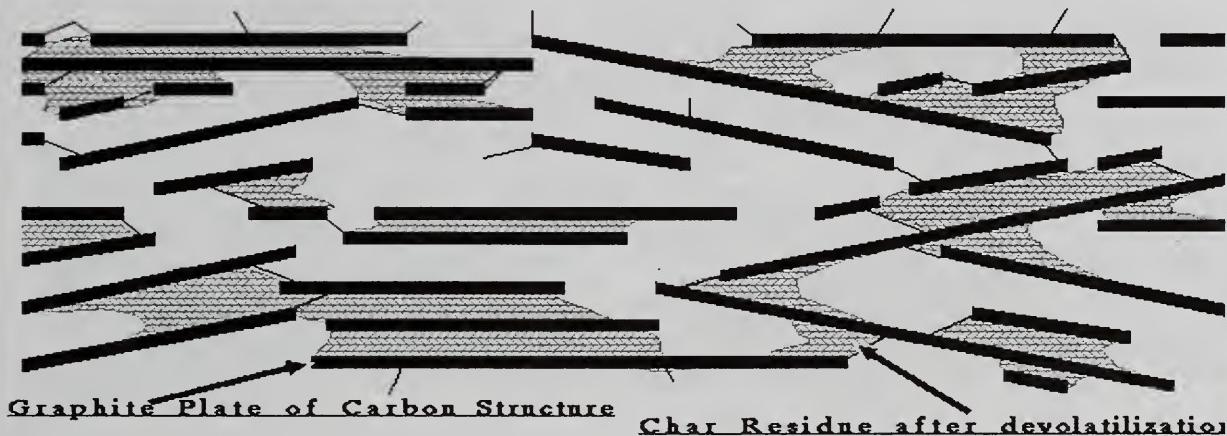
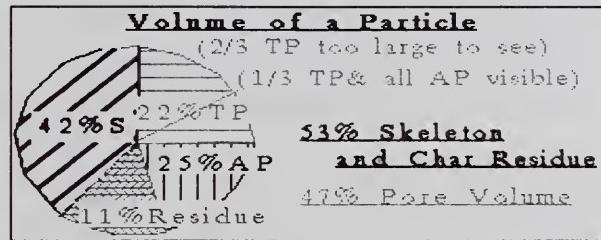


Figure 18e.

1st Cycle React Carbon
 Clean Pore Structure for
 9% Char/0.50AD Structure

0.55 g./cc AD

820 Iodine No., 230 Molasses No.

0.38 cc/g Adsorption Pore Volume

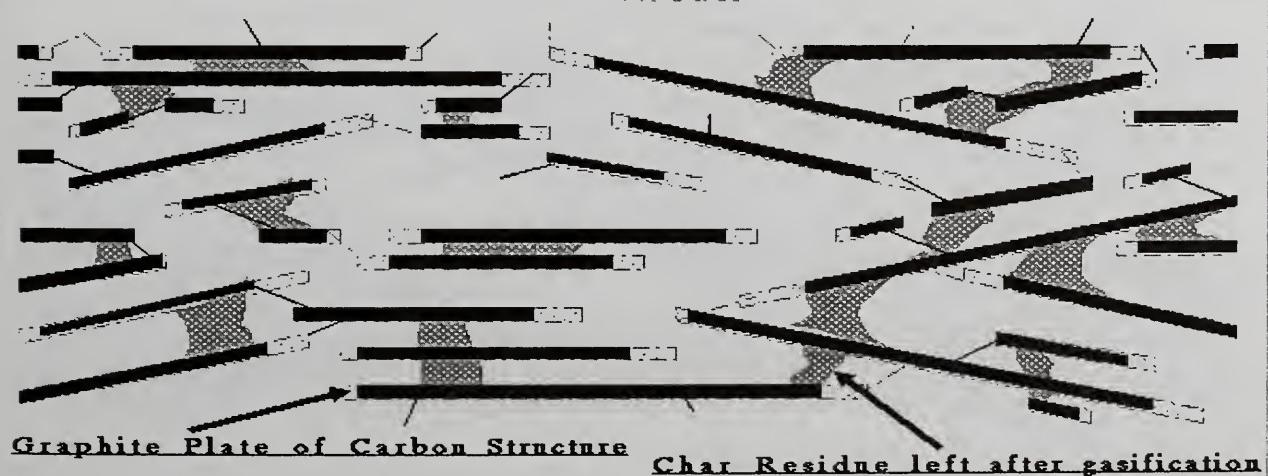
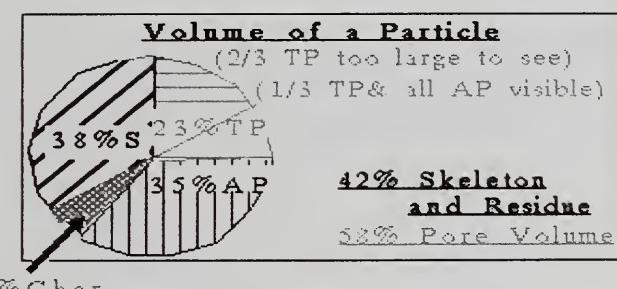


Figure 18f.

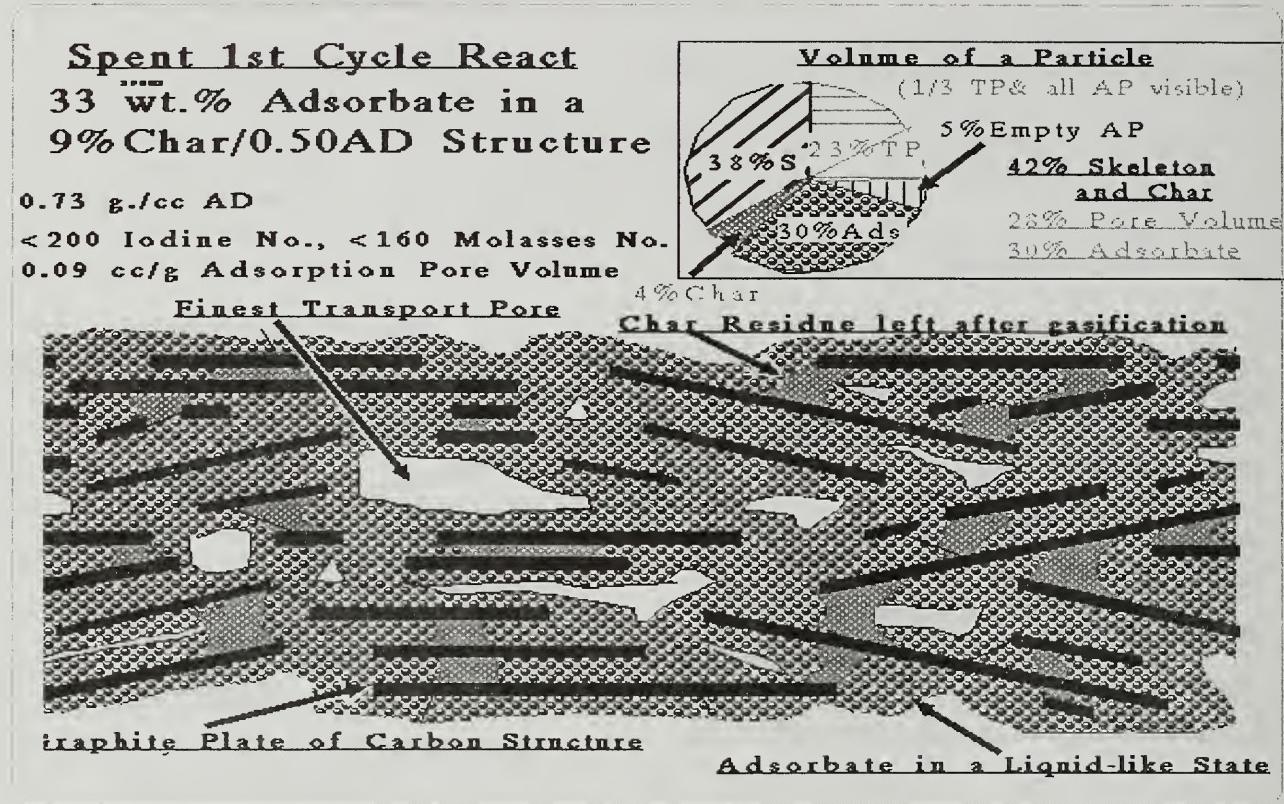


Figure 18g.

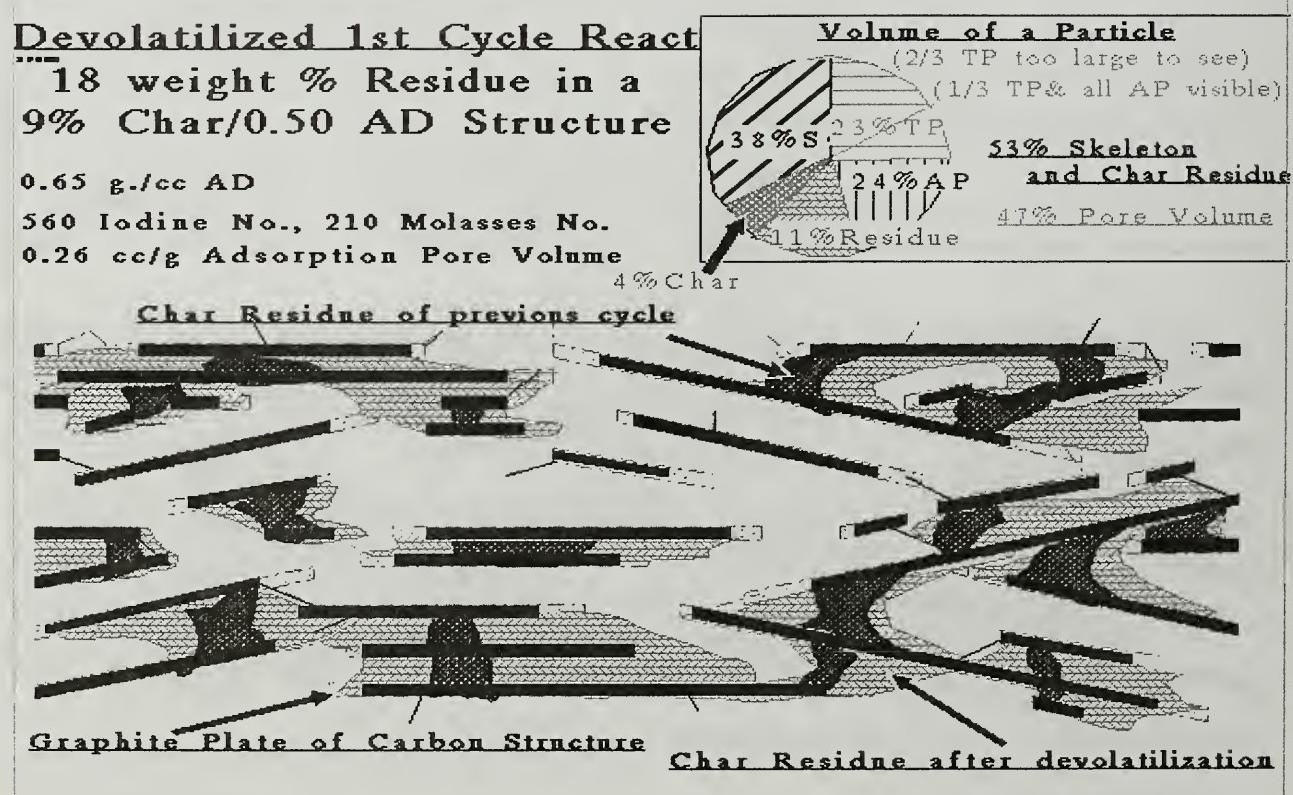


Figure 18h.

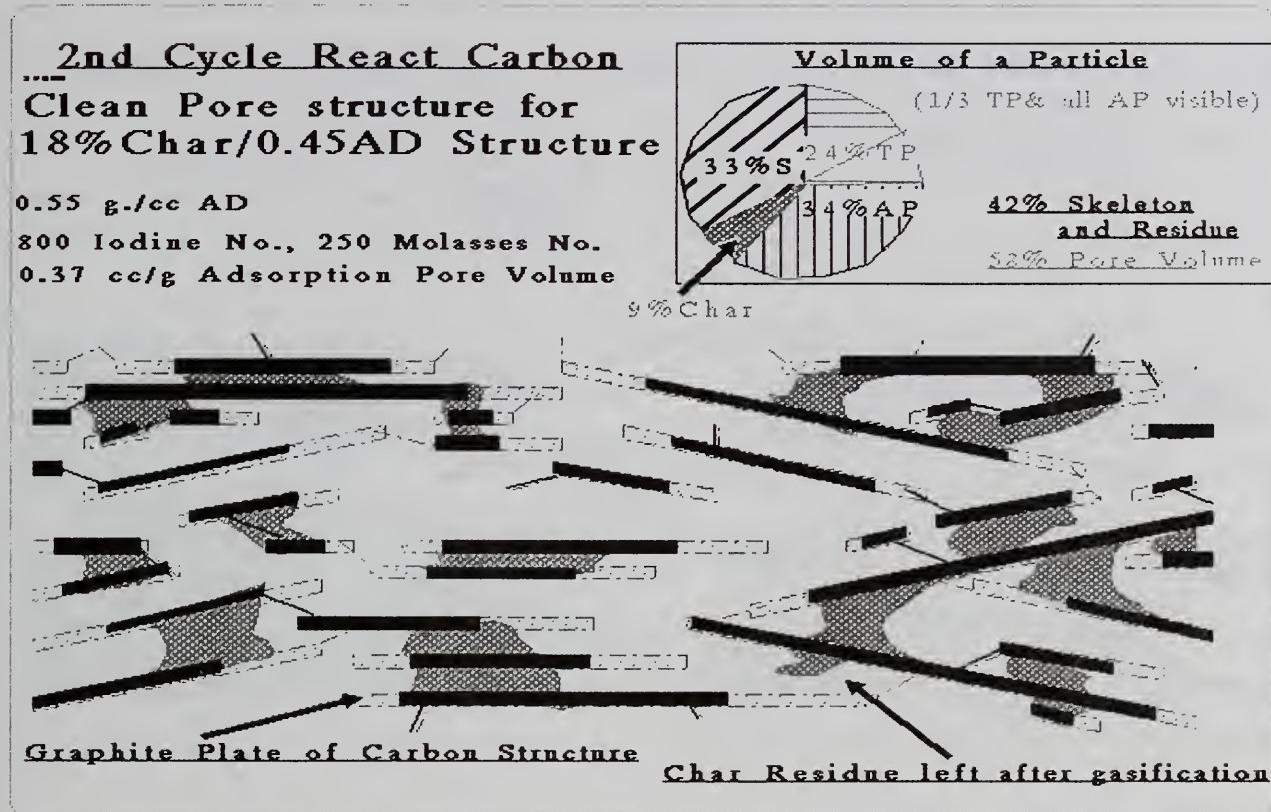


Figure 18i.

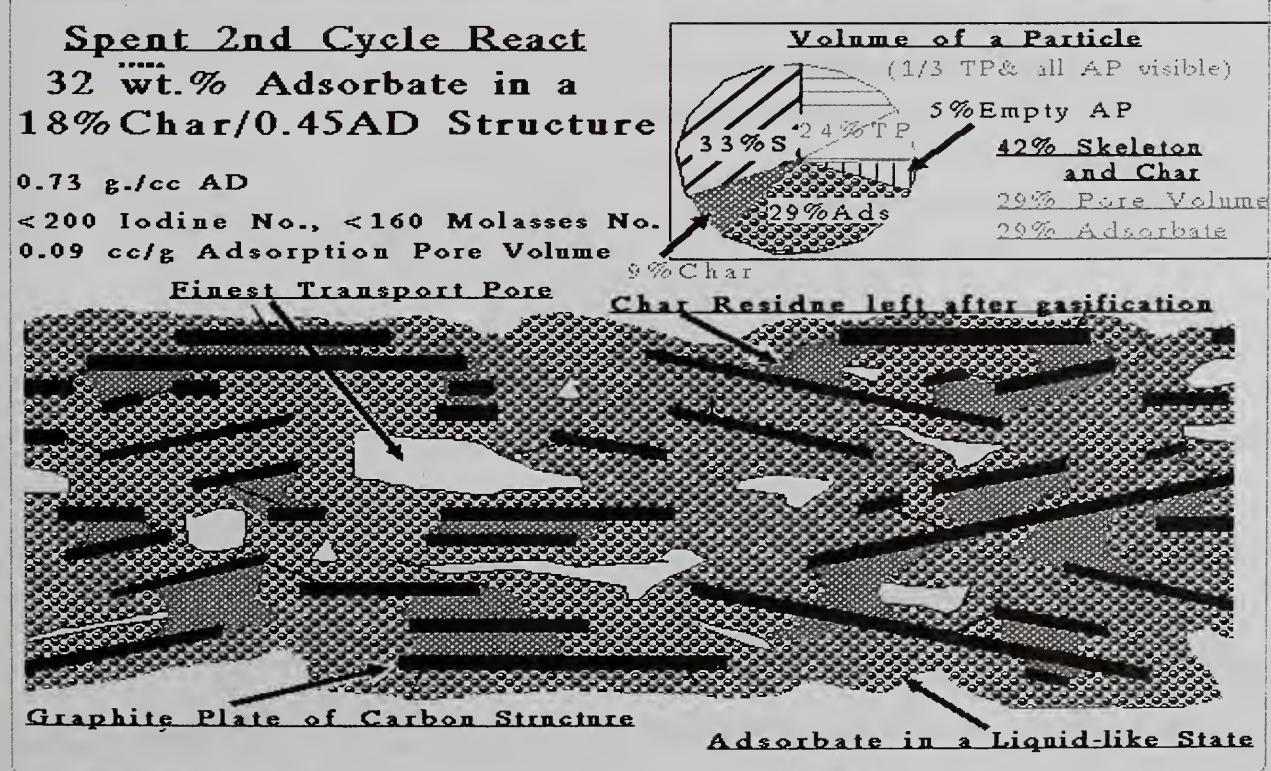


Figure 18j.

Devolatilized 2nd Cycle Reactor

**18 weight % Residue in a
18% Char/0.45AD Structure**

0.65 g./cc AD

540 Iodine No., 230 Molasses No.

0.25 cc/g Adsorption Pore Volume

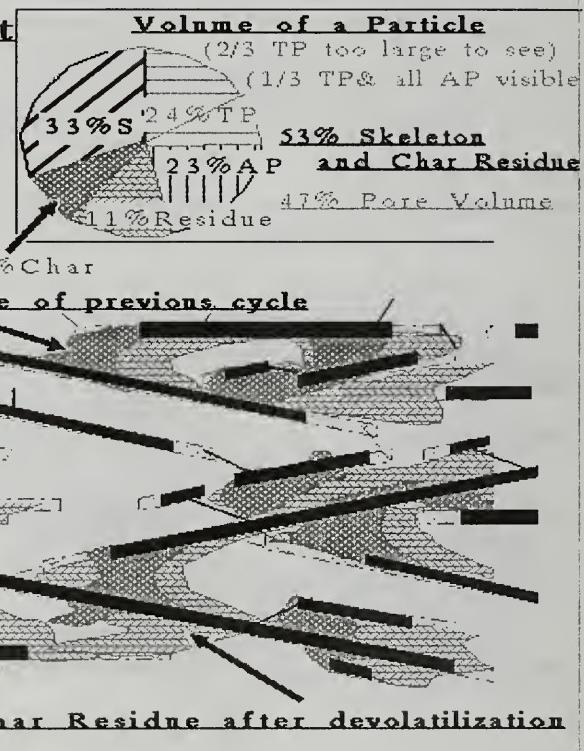


Figure 18k.

5th Cycle React Carbon
**Clean Pore structure for a
42% Char/0.32AD Structure**

0.55 g./cc AD

660 Iodine No., 400 Molasses No.

0.29 cc/g Adsorption Pore Volume

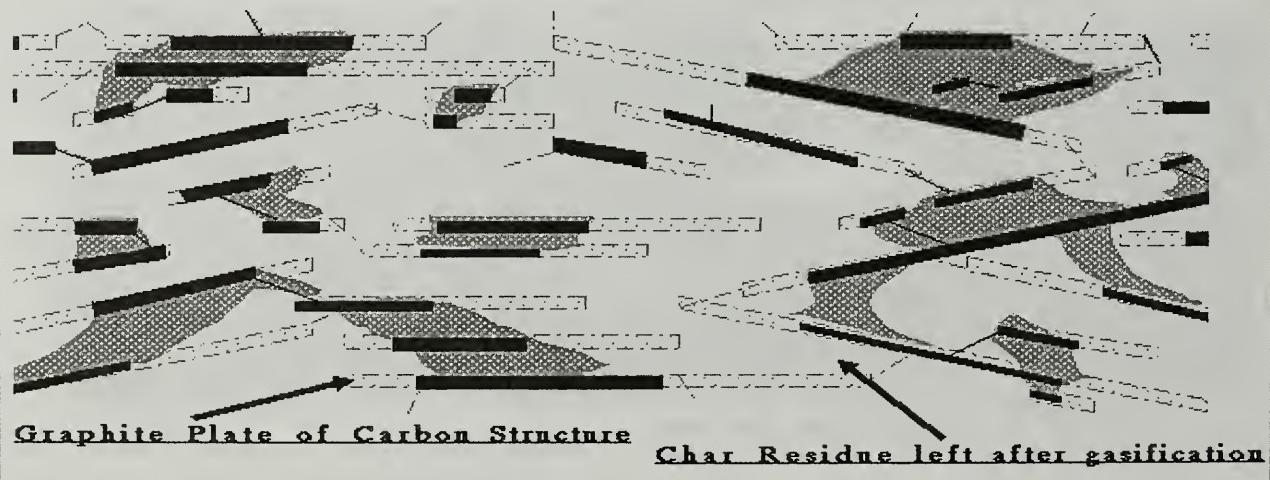
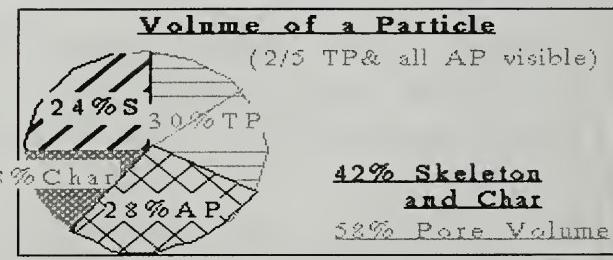


Figure 18l.

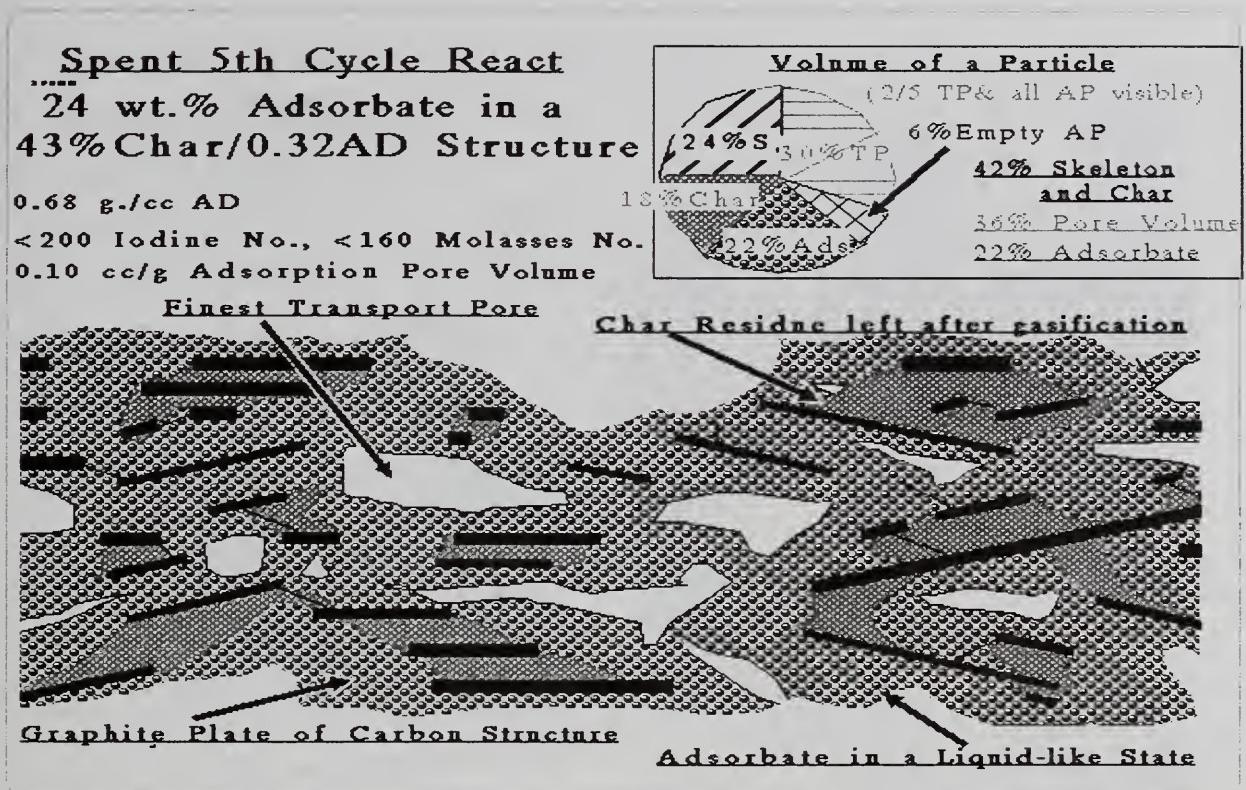


Figure 19a.

Adsorption Sites on Virgin FS200 Carbon Distribution of Adsorption Forces for Butane

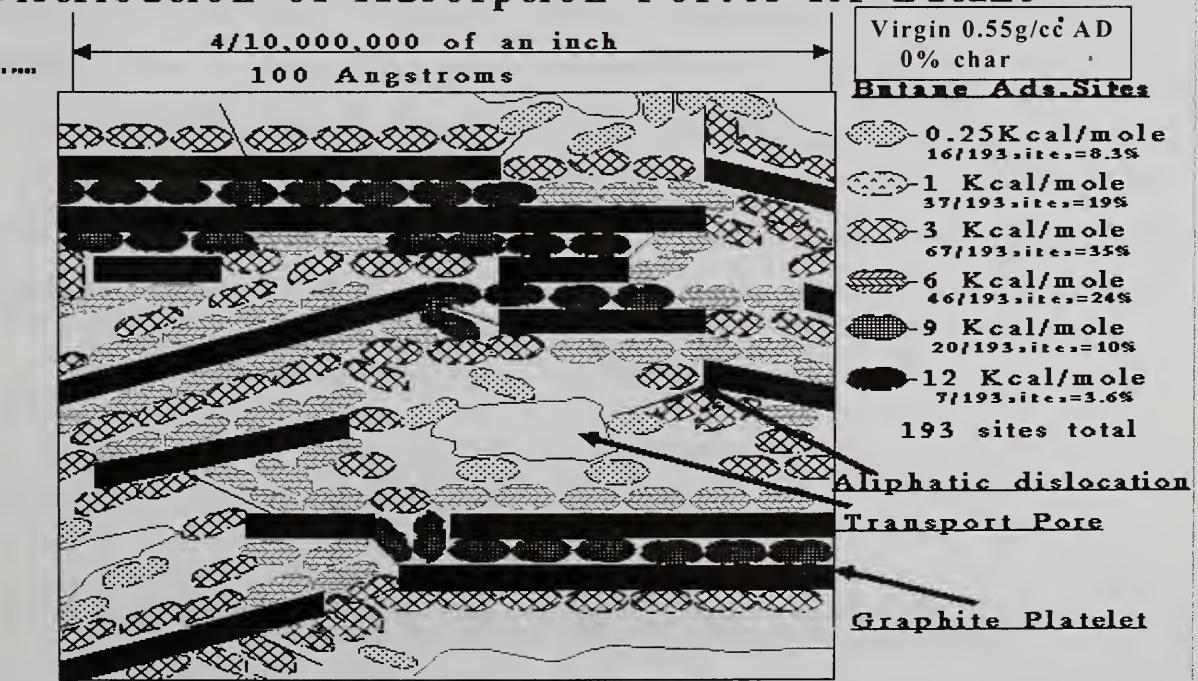


Figure 19b.

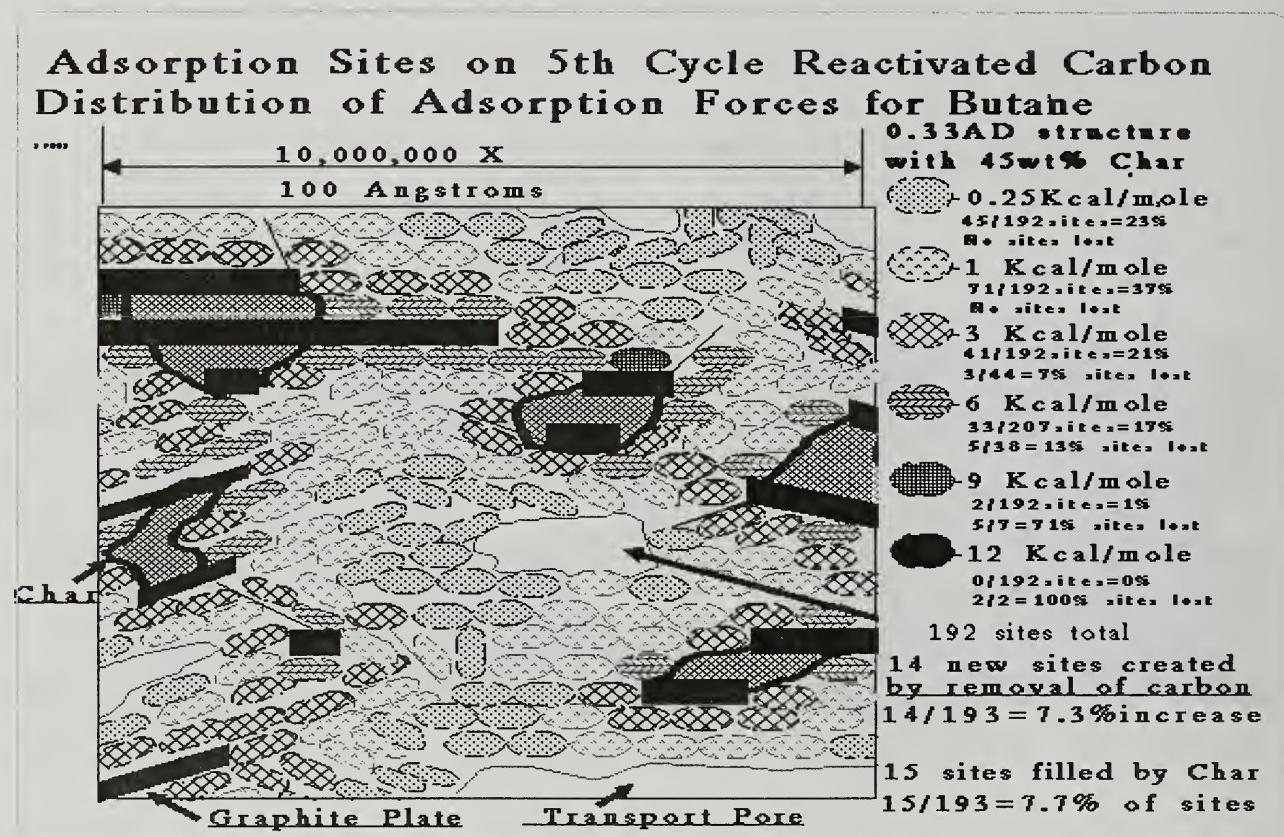


Figure 20.

What happens during Reactivation?

Physical Effect of Gasification on the Carbon /Char Structure

Initial structure is defined by the Spent Virgin Carbon

Gasification modifies structure /char by removing carbon

Range of carbon activities are made from one Spent Carbon

Remaining graphite plate width is life line of React carbon.

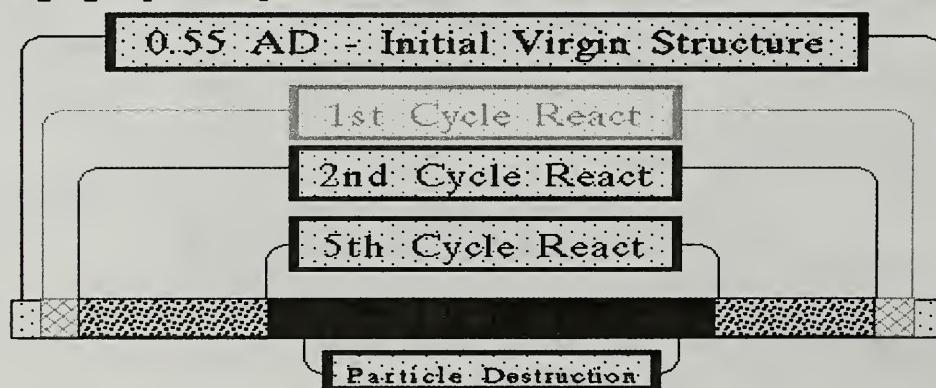


Figure 21.

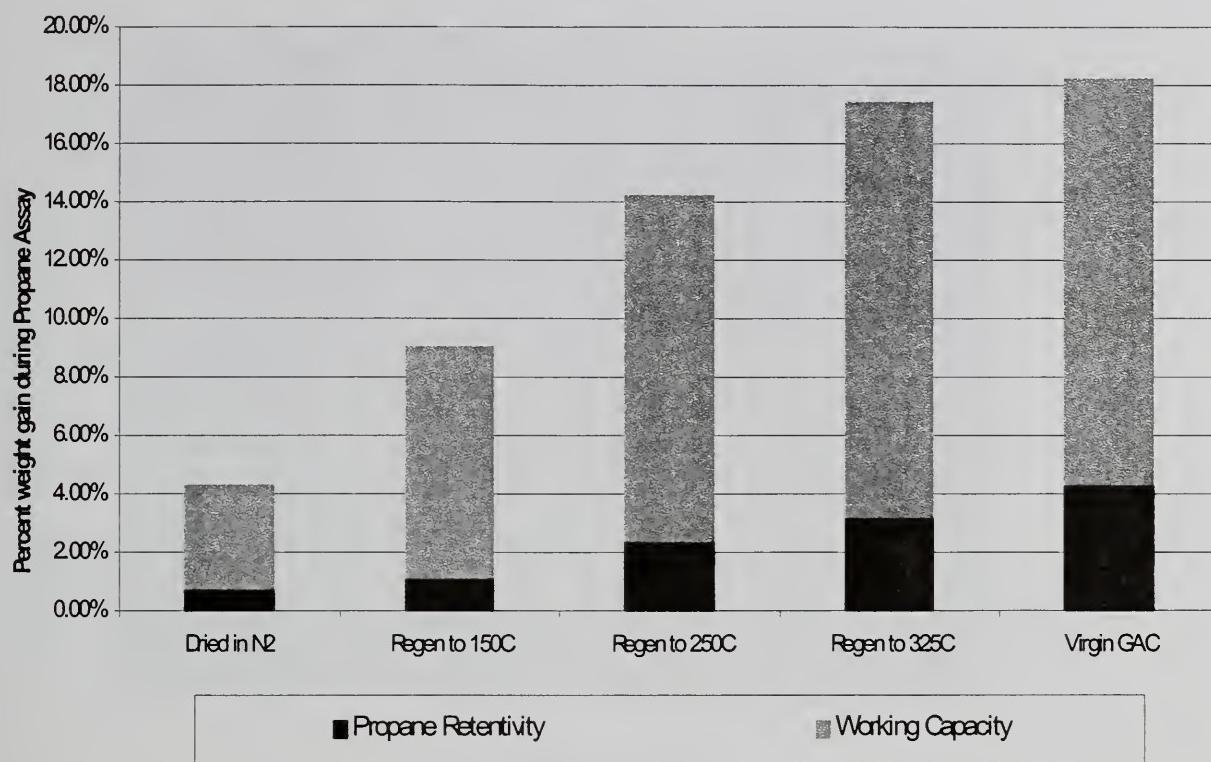


Figure 22.

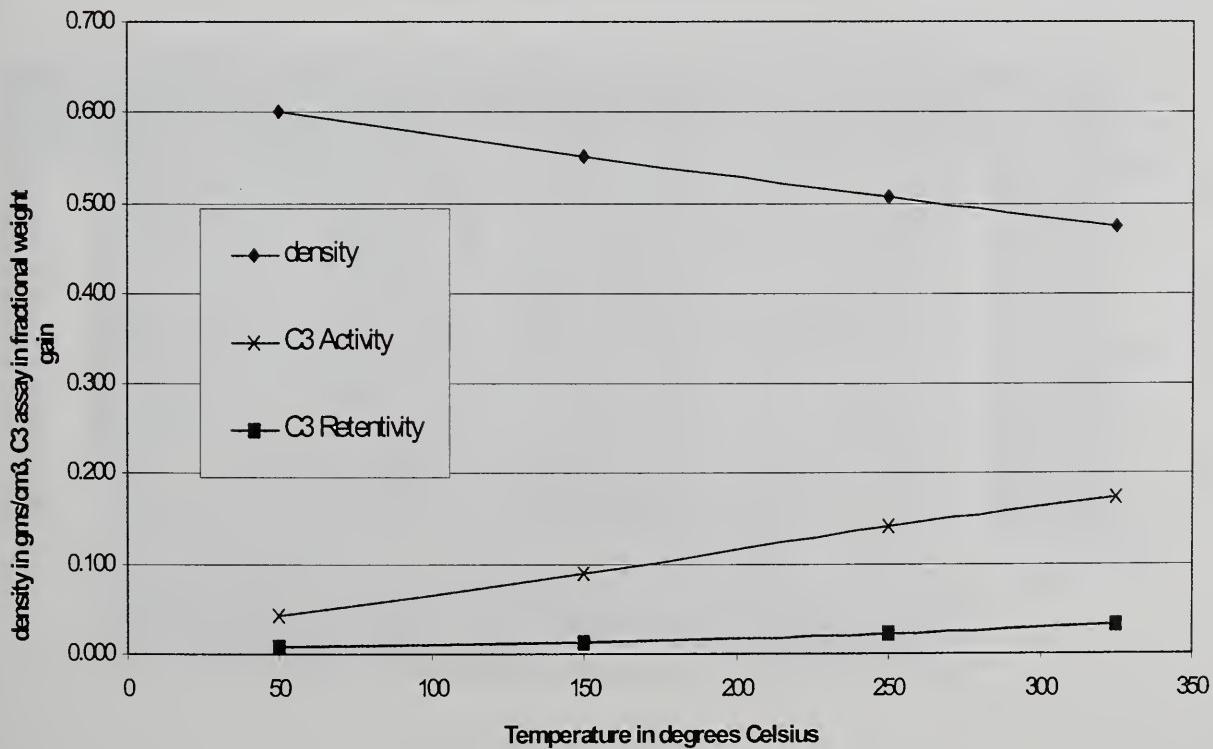


Figure 23.

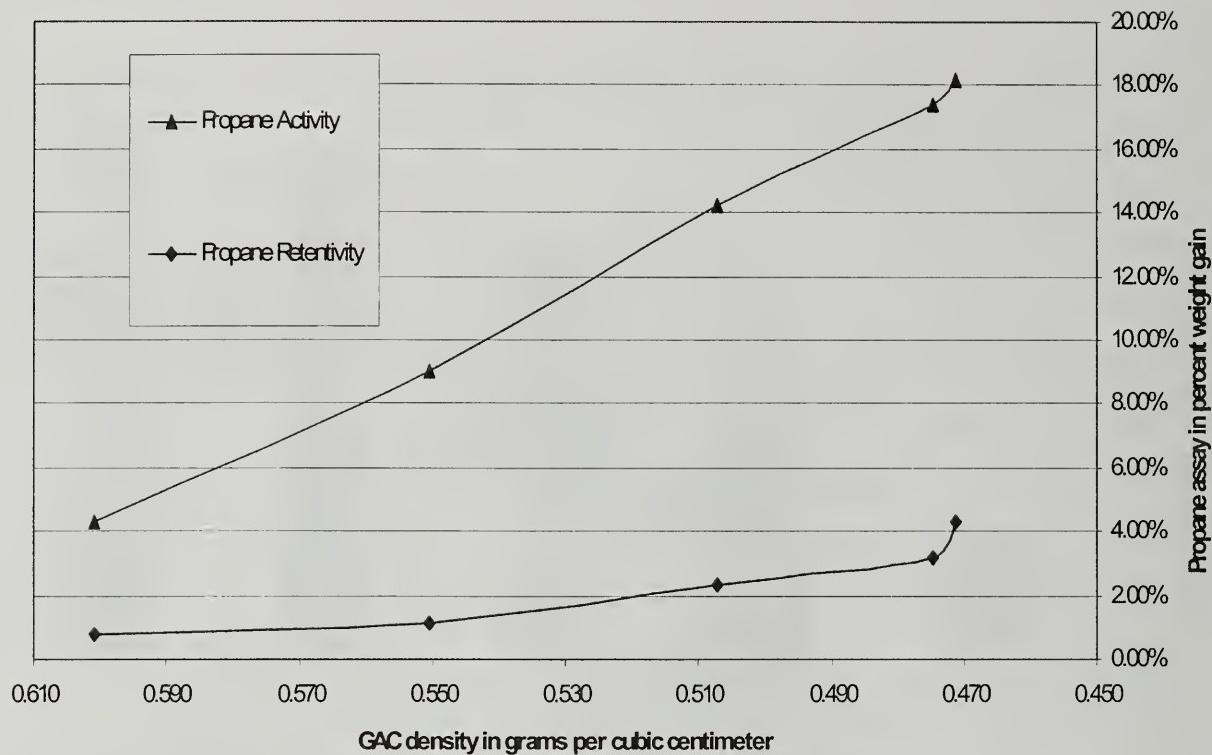


Figure 24.

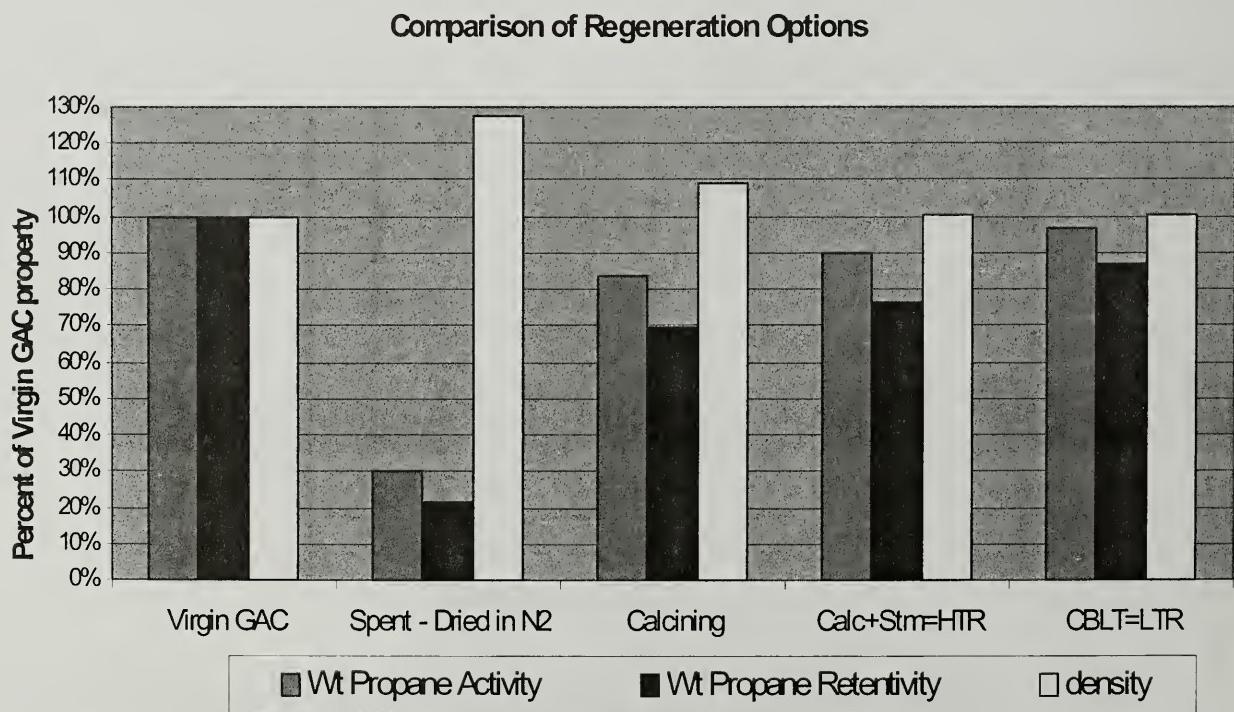


Figure 25.

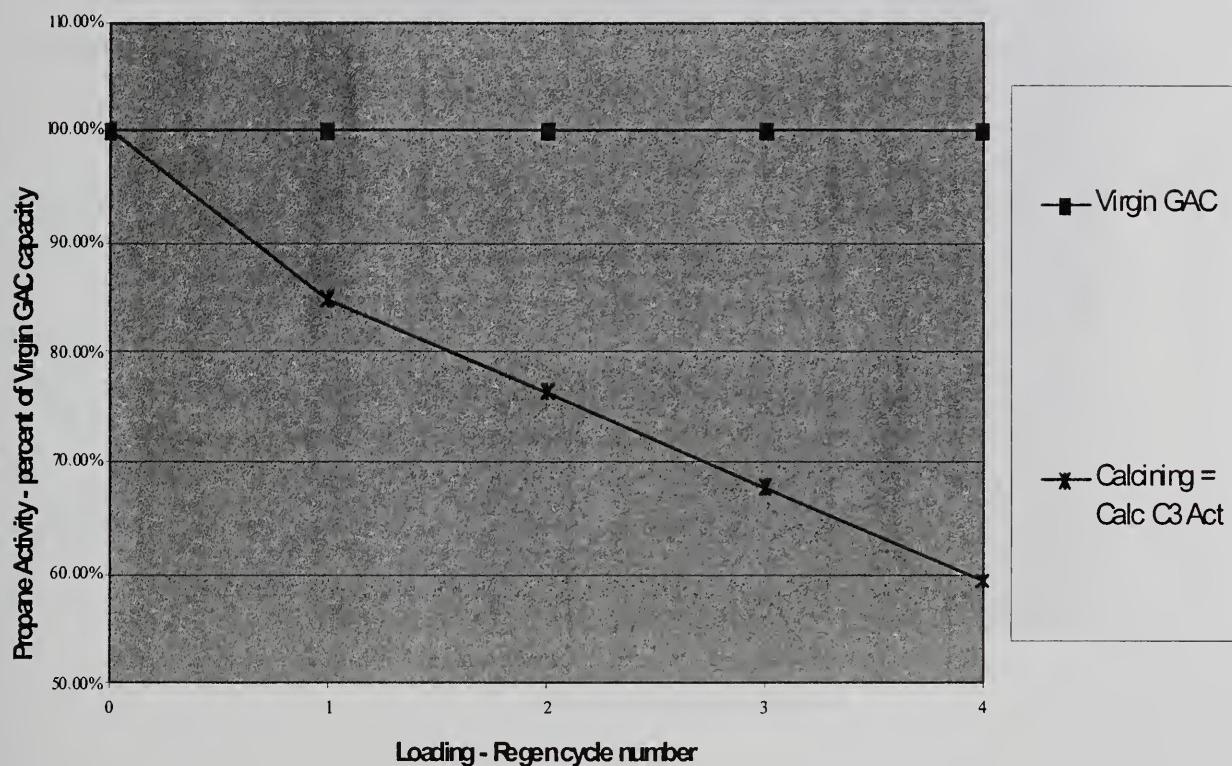


Figure 26.

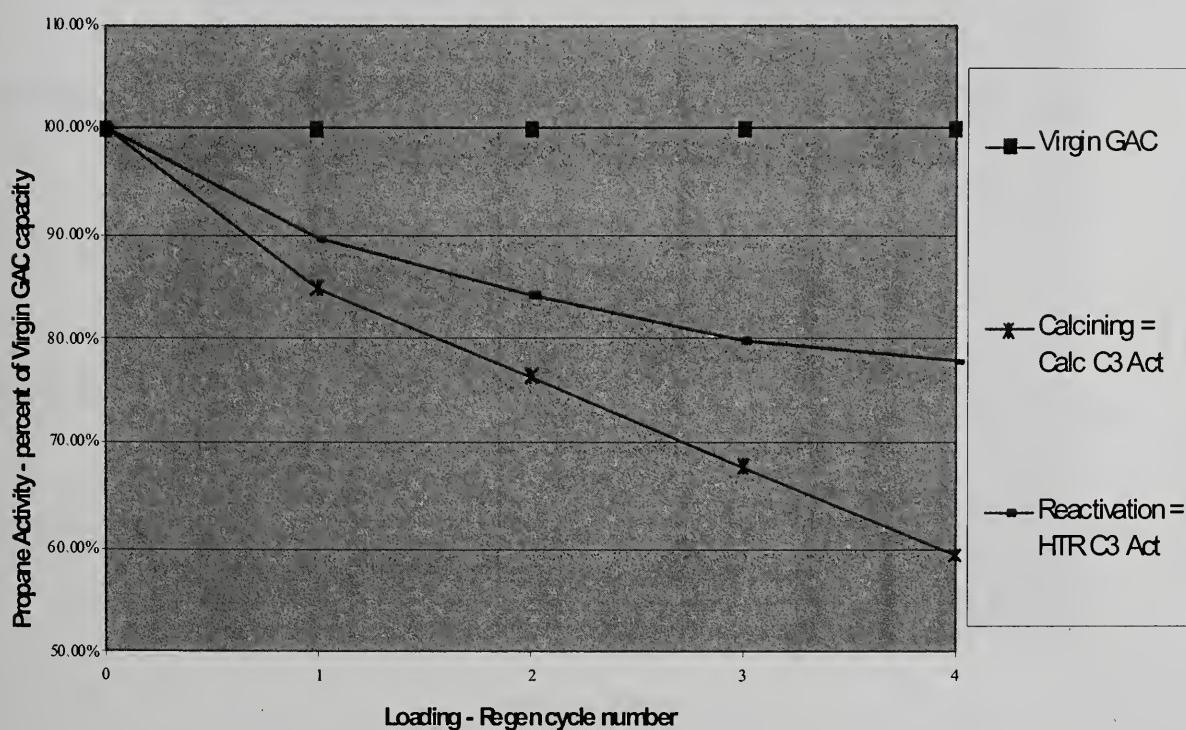


Figure 27.

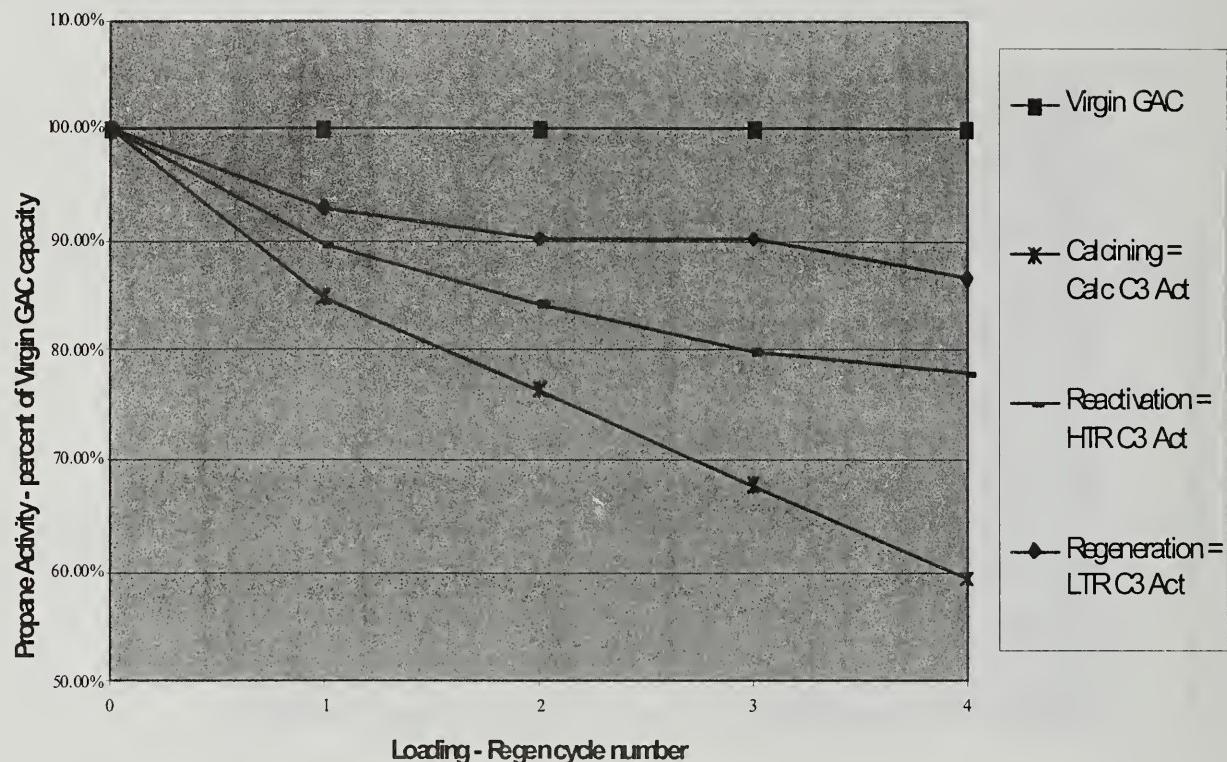


Figure 28.

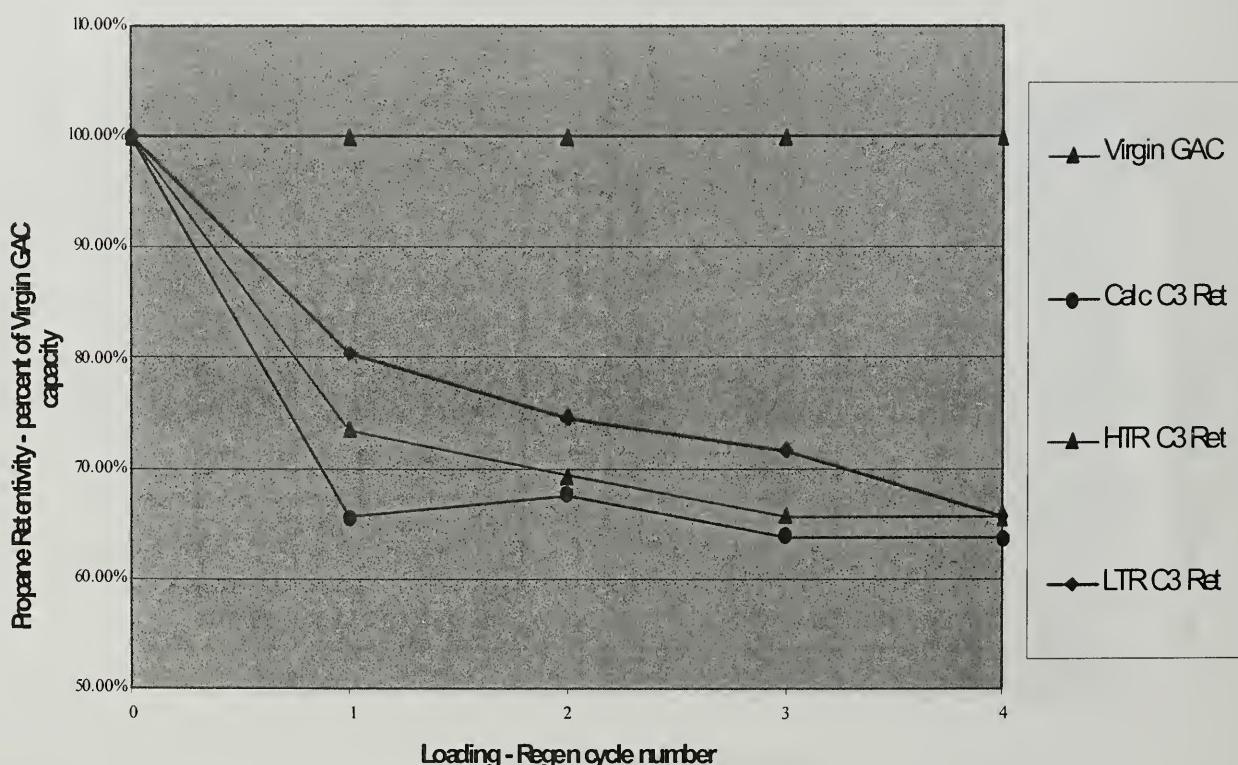


Figure 29.

CarbOxLT (LTR) vs Reactivation (HTR)

- Different Reaction Pathway & Conditions
 - HTR: Calcining adsorbates & Addn. Steam Activation
 - LTR: Surface Oxidation in air of Adsorbates
 - HTR: 850-1000 Celsius, LTR: 150-350 Celsius
 - HTR: Endothermic (add heat), LTR: Exothermic
 - HTR: raked bed <3" deep, LTR: stirred dense bed
- Different Economics of Operation
 - Capital: HTR: Refractory lined, LTR: CS/SS
 - Energy: HTR: 4,000+ Btu/#, LTR: <1,000 Btu/#
 - Losses/cycle: HTR: 3-5%, LTR: 1.5-3% (estimated)
 - Regeneration Efficiency: LTR > HTR

SUSTAINABLE TECHNOLOGIES AND VALUABLE NEW POLYSACCHARIDE-BASED PRODUCTS FROM SUGAR BEET PULP

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ABSTRACT

More effective utilization of sugar beet pulp generated from beet sugar processing can improve the economic viability of U.S. beet sugar production. Dried pulp pellet and shreds provide a nutritious and valuable animal feed co-product from pulp for both local and export markets. However, the future of such markets may be threatened by increased availability of alternative feed materials from grain-based bioethanol production and customer concern for beet pulp derived from genetically modified plants that are being developed and pending commercial release. Beet pulp represents a largely untapped source of cell wall polysaccharides such as pectin that can be recovered and converted into higher-value biobased products. New biobased products are therefore sought from beet pulp to generate more revenue for growers and processors and to replace imported and/or petroleum-based products. Research on our CRIS (Official ARS/USDA Research) project is being conducted toward developing fundamental knowledge of the enzyme and polysaccharide components of the sugar beet cell wall; creating innovative new functional food, feed, and non-food biomedical and industrial bioproducts; and establishing efficient and sustainable conversion processes for their isolation and production. Our specific objectives, capabilities, and accomplishments are summarized in this report.

INTRODUCTION

The Crop Conversion Science and Engineering Research Unit is located at the Eastern Regional Research Center (<http://www.arserrc.gov/www/>) in suburban Philadelphia. The Center was opened in 1940, one of four regional research laboratories of the U.S. Department of Agriculture. There are six research units located at the Center, which are distributed generally between agricultural product utilization and food safety programs conducting fundamental, applied and

developmental research on agricultural commodities. The Center employs about 100 senior scientists and engineers, 120 scientific support staff, and about 80 administrative and facilities support staff. The mission of the Crop Conversion Science and Engineering Research Unit is to conduct research leading to the development of new processes for the conversion of low-valued crops and food processing by-products into valuable biobased products and biofuels. In the Research Unit, there are four funded CRIS projects (<http://cris.csrees.usda.gov/>) with research conducted under National Programs 306 (www.ars.usda.gov/research/programs.htm), Product Quality and Utilization of Agricultural Products, and 307, Bioenergy and Energy Alternatives:

- Sustainable Technologies for Polysaccharide Based Functional Foods and Biobased Products (M.L. Fishman, et al.)
- New Biobased Products to Increase Demand for Grains and Grain Byproducts (R.A. Moreau, et al.)
- Enzyme-Based Technologies for Milling Grains and Producing Biobased Products and Fuels (D.B. Johnston, et al.)
- Improving the Economic Competitiveness of Ethanol Production (K.B. Hicks, et al.)

Further information on the projects, including objectives, staff, and annual reports can be found at the local (www.arserrc.gov/ccse/) and National Program (www.ars.usda.gov/research/) websites.

Primary stakeholders for the project Sustainable Technologies for Polysaccharide Based Functional Foods and Biobased Products include the U.S. beet sugar and citrus juice industries. These industries are under challenge by a number of economic forces, including increasing foreign competition and continuing low commodity prices. These industries generate tens of millions of tons of processing residues. Such byproducts are sold as a nutritious animal feed (Fadel et al., 2000). Though of low unit value (ca. \$85/ton), they are important for overall profitability by recovery of energy cost inputs required to dry the residues for stabilization and shipping. This co-product market faces competition by increasing availability of other domestic feed products, in particular the distillers dried grain solids from corn generated from bioethanol production. To improve the economic viability and competitiveness of U.S. beet sugar and citrus juice industries, we are working to develop new and more valuable bioproducts from these plant processing residues.

BACKGROUND – PECTIN

The residues from sugar beet and citrus processing largely represent the cell walls of root and fruit tissues, respectively. Plant cell walls are composed predominantly of the structural polysaccharides cellulose, hemicelluloses, and pectins (Brett and Waldron, 1996). Each polysaccharide type is distinguished by chemical, physical, and functional properties, which generally define how they are isolated and used technologically. Their effective utilization, however, is largely dependent on their efficient extraction and separation, on whether the isolated polysaccharides can be used directly or require further processing, and on whether they provide unique functionality not otherwise available with synthetic polymers.

Citrus peel and beet pulp are notable for being particularly rich sources of pectin. Pectin is a valuable food hydrocolloid traditionally used as a gelling agent in jams and jellies. Food grade pectin is commercially extracted from apple and citrus fruit tissues. General information on commercial pectin is available at the International Pectin Producers Association website (www.ippa.info/what_is_pectin.htm). In the U.S. pectin sells for about \$6 to \$10 per lb. Production and use of pectin as a food hydrocolloid is defined by Codex (1986) (and adds to the cost of pectin). However, the market for pectin in traditional food applications is rather mature, while the potential pectin production from U.S. citrus peel and beet pulp is in huge excess over market needs. Further, only the highest quality feedstock is used for pectin production (meeting specifically defined chemical and functional properties). It should also be noted that there is no longer significant domestic production of pectin, despite the abundance of citrus peel and related material. Therefore it is imperative to identify and develop alternative non-food uses of polysaccharides, such as pectin, that may be recovered from processing residues. Various uses proposed and investigated have been reviewed (Thibault and Bonnin, 2000; Endress, 1991).

Pectin is generally composed of three related polysaccharides based on 1,4-linked α -D-galactosyluronic acid represented by 1) homogalacturonan that is variably esterified with methanol (C6 carboxyl esters) and acetic acid (C2 and C3 hydroxyl esters), 2) rhamnogalacturonan-I, which contains arabinose and galactose-containing side-chains variably attached at the C4 position of rhamnose, and 3) a highly conserved subunit called rhamnogalacturonan-II (Ridley et al., 2001). Pectin isolated from beet root cell walls varies considerably in its organization, composition, and physical/chemical properties compared with that isolated from fruit cell walls. These differences can be interpreted to reflect different structural and functional adaptations of the beet root as an underground storage organ. Fruits that soften during ripening processes are undergoing "programmed" disassembly of cell wall polysaccharides (Brownleader et al., 1999). During root growth, the cell walls continue to undergo cross-linking modifications to increase rigidity for mechanical support and to reduce digestibility by underground pests and microorganisms. This structural rigidity creates a technological challenge for effective extraction of individual polysaccharide classes from the cell wall matrix. However, the distinctive chemical features of beet root polysaccharides also provide opportunities for creating new biobased products.

Compared to citrus fruit pectin, sugar beet pectin isolated from beet pulp has a reduced galacturonic acid content (<65%), lower molecular weight, reduced degree of methylesterification, and a high degree of acetylation (Fishman and Jen, 1986). These features are considered primary contributors for the poor gelling properties of beet pectin in traditional food applications. Acetylestes, like methylesters, strongly influence solubility properties of sugar beet pectin and their susceptibility to depolymerizing enzymes. Chemical features distinguishing sugar beet pectin include the high content of arabinan and arabinogalactan, which is associated as branch structures on rhamnogalacturonan. These neutral polysaccharides can represent 20% or better of the total cell wall polysaccharides present in beet pulp, and they are responsible for the unusually high water adsorbing capacity of beet pulp. Unique in dicot plants is the presence of ferulic acid that is esterified to arabinose and galactose in the cell wall. Covalent cross-links occur between ferulic acids associated with pectin molecules. They may also cross-link to extensin or other cell wall proteins. There are at least seven distinct (acetyl-, feruloyl- and methyl-) ester linkages present in pectin structure

(Williamson et al. 1998), and each may require a corresponding enzyme to hydrolyze them. Sensitive and specific analytical tools are needed to measure these esters and to detect these enzymes. These and other enzymes may be used to efficiently release pectin in new extraction technologies to isolate pectin and other cell wall polysaccharides and define their structure-functional property relationships.

CRIS RESEARCH PROJECT

Fundamental research in this project (www.arserrc.gov/ccse/SustainableTechnologies.htm) provides new knowledge in compositions, structures, and functional properties of cell wall components in beet root and citrus fruit cell walls, and this is driven by analytical and separations chemistry. These fundamentals are applied to develop physical, chemical and biocatalytic technologies useful for the efficient and sustainable extraction and preparation of pectin, its derivatives, and related cell wall polysaccharides that can be used to create biobased industrial products, functional food ingredients and nutraceuticals, and valuable new biomaterials. This project is implemented by four senior research scientists, providing multidisciplinary capabilities in polysaccharides and biopolymers, sugars and oligosaccharides, proteins and enzymology, and biomaterials. This is further complemented with a process engineering support group staffed with cost, chemical and mechanical engineers. The expertise and approaches led by the senior scientists on the project are summarized here:

Research directed by Marshall Fishman (physical and polymer chemistry) has focused on the solution and solid state interactions of hydrophilic macromolecules such as pectin. This has provided a better understanding of the role of pectin as a soluble dietary food fiber and as a cell wall component involved in the post harvest ripening of fruits. The solution properties of pectin have been characterized by high performance size exclusion chromatography with concentration, dynamic and static light scattering and viscoelastic detectors, by membrane osmometry, and by end-group titration. In close collaboration with Peter Cook of the ERRC Microscopy Imaging facility (www.arserrc.gov/ctmi/), pectin aggregation properties have been imaged by electron microscopy, and the nanostructure of native pectin sugar acid gels has been imaged by atomic force microscopy. New film materials have been developed from cell wall polysaccharides. For this, the mechanical, solubility and microstructural properties of pectin/starch/glycerol (P/S/G) edible films and pectin/polyvinyl alcohol/glycerol biodegradable films have been characterized and patented. New extraction technologies have been developed to lower the costs and improve quality of pectin from U.S. orange peel. A microwave-based pectin extraction method, under rapid heating with constant pressure, has been patented, and a new pilot-scale process using continuous steam injection for extraction of cell wall polysaccharides and generating bioactive oligosaccharides is in development. Current research is now focusing on applying extraction technologies to beet pulp and characterizing the pectin and hemicelluloses isolated from this material.

Research directed by Arland Hotchkiss (plant physiology and carbohydrate chemistry) has focused on analytical and preparative separations of oligosaccharides. High performance anion-exchange chromatography with pulsed amperometric detection has been used extensively to develop methods to separate and detect oligosaccharides with defined degree of polymerization (DP) up to 50, and isolate individual oligosaccharides up to DP 20, which have been provided to

collaborators in academia and industry. These methods are also used to define depolymerization patterns of pectinases. Current research is now focused on developing second generation prebiotics from orange peel and citrus pulp oligosaccharides. Prebiotics represent non-digestible polysaccharide fragments that, when consumed, can promote the growth of health-promoting human gut bacteria while limiting the growth of pathogenic ones. Prebiotics may also act to block toxin binding in the gut, thereby inhibiting their action. Oligosaccharides generated by a new thermomechanical process are now being isolated to evaluate their bioactivity. This may provide new nutraceutical products for humans and feed components for animals.

Research directed by Brett Savary (plant physiology and cell wall biochemistry) has focused on developing enzymes that modify the structure of polysaccharides to improve end-use functional properties. Enzymes such as pectinases have been purified and characterized using protein chromatography technologies such as the Biocad 700E Protein Chromatography Workstation and an array of electrophoresis tools. Considerable effort has been directed to produce well-defined monocomponent orange pectin methylesterases and use them to manipulate charge density and distribution of free galacturonic acid in pectin. Such modified pectins have been prepared in gram quantities using an enzyme-reactor system. The structural and functional characterization of modified pectin has been conducted in collaboration between project scientists with ARS scientists in Winter Haven, FL. Such modified pectins may be used in new food applications as stabilizers and as templates for subsequent construction of molecular scaffolds for biomedical applications. Recent research has resulted in the development of an innovative new gas chromatography-mass spectrometry method useful for simplified and sensitive determination of polysaccharide ester contents and measuring esterase activities. This method is described in more detail elsewhere in these Proceedings (Savary and Nuñez). New research has also commenced in identifying and isolating new polysaccharide-modifying enzymes present in sugar beet cell walls using proteomics and separations instrumentation recently acquired at the ERRC. The centerpiece is the ABI 4700 proteomics analyzer (MALDI-TOF/TOF), and this is supported by state-of-the-art multidimensional protein LC and capillary/nano-LC systems.

Research directed by LinShu Liu (polymer chemistry and materials science) has focused on engineered polysaccharide materials for biomedical applications. Pectin and other biopolymers have been modified and micro/nano-particles and three-dimensional tissue scaffolds fabricated. Because they are biocompatible and biodegradable, they are being investigated for use as drug carriers, such as colon-specific drug delivery, and as a matrix for tissue engineering. The biomaterials generated form porous structures that can be further modified with interpenetrating networks to manipulate chemical and physical functionality. These materials appear as "microsponges" under the microscope. New uses are currently being investigated for controlled-release of cosmetics for personal care, nutrients and pesticides for plant growth or protection, and construction of "smart" polymers with self- and media-regulated functionality in response to applied stimuli. Other research is also being conducted in cooperation with M. Fishman using ERRC extrusion and rheology resources (www.rrc.ars.usda.gov/cepr/) for fabrication of new materials from beet pulp and citrus peel. Extruded and food-grade films are being generated and analyzed with dynamic mechanical testing and texture analysis.

Engineering resources in the Research Unit and available to this project are directed by Kevin Hicks (Research Leader, carbohydrate chemistry and biochemistry). This includes the SUPER

(Scale-Up, Process and Economics Research) Support Group and a Cost Analysis Team. This represents the Agency's only process engineering unit with the facilities and expertise to do pilot plant research involving basic theoretical investigations, process development, scale-up, simulation and economic feasibility studies. Engineering resources include a pilot plant facility for new process scale-up, which can provide process cost data such that cost analyses can be performed to direct research efforts within a realistic economic framework.

RECENT ACCOMPLISHMENTS RELEVANT TO BEET PULP UTILIZATION

Recent research accomplishments from this project that are particularly notable for sugar beet pulp utilization include:

- Produced high molecular weight moderate viscosity pectin from unheated sugar beet pulp using patented microwave-based extraction process.
- Demonstrated pilot-scale thermomechanical process for polysaccharide extraction and the isolation of oligosaccharides in gram quantities from citrus and beet pulp for prebiotic studies.
- Developed preparative oligogalacturonan purification to DP 20.
- Prepared well-defined monospecific pectin methylesterases and applied to produce calcium-sensitive pectin without degrading molecular weight.
- Established new analytical method to greatly simplify determination of methanol and acetic acid in sugar beet pectin and processed pulp.
- Fabricated new pectin-based composites for biomedical applications.
- Evaluated modified sugar beet pulp as a filler in phenol-formaldehyde glue mixes for plywood.

Please see our local and ARS website for our annual reports and publication lists from our research (www.ars.usda.gov/research/programs/programs.htm?NP_CODE=306).

Our goal is to work with industry stakeholders and customers to develop, transfer, and apply bioproducts and processes that can enhance competitiveness, generate new revenues, and support the rural economy. For this we seek to find alternative uses of beet pulp as an animal feed to new ones that will increase the value and demand for this renewable resource. To meet this goal, certain issues and realities must be considered. These include the need to place new technologies at the site of "feedstock" generation and to generate multiple products, applying the biorefinery concept (Morris and Ahmed, 1993). Because of the seasonal nature of the sugar production campaign and costs for distant transport and storage of processing residues, new technologies must be located at or close to the sugar factory and be integrated with existing facilities and processes. This suggests a modular nature for new technologies, providing process integration and scale-down. This can allow flexibility for feedstock availability and changing market needs. New co-products produced at the factory should allow capture of added value and benefit the local/rural economy. Multiple products, however, must be identified and developed, and waste

streams eliminated. Generally, valuable functional polysaccharides may be produced for industrial applications, with the secondary extractables being directed to fermentation processes for biofuel and biochemical production, and final solid residues used as inert fillers or perhaps hydrogen fuel generation. Supportive public policy and a greater commitment to research with closer cooperation between producers, ARS, and product end-users are needed. Such cooperation should help reduce risk and to focus research activities to develop products to meet market needs.

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COMPOSITION AND STRUCTURE OF CELL WALL POLYSACCHARIDES FROM SUGAR BEET GROWN UNDER MEDITERRANEAN CLIMATE AND RELATION WITH BEET PROCESSING

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ABSTRACT

Cell wall polysaccharides were isolated as alcohol-insoluble solids (AIS) from Moroccan sugar beet, wet pulps, pressed pulps and diffusion juice at two stages of the harvest season (Early and Late samples). Pectins and hemicelluloses were extracted from beet slices and pulp by a sequential treatment with water, CDTA, hot dilute hydrochloric acid, cold dilute sodium hydroxide, cold 1 M and 4 M sodium hydroxide, and finally water at pH 7. Pectins were also extracted from sugar beet brei by hot water under conditions simulating the sugar factory. The highest extraction yields were obtained for the alkali and dilute acid treatments. Pectins extractable with water still remained in the pressed pulps (1.5 % of AIS). Composition of the pectins and hemicelluloses extracts was determined and the purification carried by ion exchange chromatography. Percentages of methylation of pectins were determined. Phenolic acids associated with the cell wall polysaccharides of pressed pulp were also extracted and analysed by HPLC.

Marked differences in yield and composition of beet polysaccharides can be obtained in relation to the variety and the growing site of crop. The polysaccharides precipitated with alcohol from

factory diffusion juice were mostly pectic in nature and the neutral sugars were composed mostly of arabinose, galactose, glucose and rhamnose.

The extraction and degradation of pectins in the sugar factory depends on the processing parameters. The level of pectins degraded under hot alkaline conditions during the purification step in the factory can be determined by measuring the amount of total galacturonic acid in diffusion and thin juices. It was shown on model solutions that the presence of monomers of galacturonic acid in the factory juice increases the colour formation and affected negatively the processing performances.

AIS of Moroccan sugar beet pulp was very rich in xylose, but had lower arabinose and uronic acid contents. These characteristics and the content of ferulic and diferulic acid could be used to give an added value to the pressed pulp which is still used as animal feed only.

INTRODUCTION

Sugar beet is grown in Morocco under Mediterranean climatic conditions of low rainfall and high temperature and sunshine. Under irrigation, the mean average yield of sugar beet roots is about 50 t/ha and yield of sucrose is about 160 mg/g. The period of sowing depends on the area, climate and beet variety, but usually this period is from October to January and the sugar harvest starts in the middle of May to the beginning of June under high summer temperatures. This method of operation causes a rapid degradation of beet quality with sucrose inversion [1, 2]. Given a high level of inverted sugar and sodium ions [3, 4], the "technological value" of the sugar beet grown in Morocco, is the same as in other Mediterranean countries (south of Italy, south of Spain and Greece), but it is different from beet quality in Northern Europe.

Polysaccharides are another factor in beet quality; they are considered as non-conventional elements of beet quality, but their effect on beet quality and beet processing is not sufficiently defined. No data are available on the polysaccharide composition of sugar beet grown in Morocco. The Moroccan climate conditions may influence the composition and the properties of the sugar beet polysaccharides. These macromolecules can also play a role in sugar beet processing, especially during filtration and can be involved in colour formation [5, 6]. We have therefore undertaken this study to characterise the sugar beet polysaccharides in sugar beet slices, diffusion juice and in pulp.

Many authors [e.g. 7, 8, 9] have studied the pectins of sugar beet grown in Northern Europe. However, few data [7, 10, 11] on cell wall pectins from sugar beet slices are available, in contrast with the abundant literature on beet pulp [8, 9, 10, 12, 13, 14] and little work has been done on beet hemicelluloses [15, 16, 17]. Some works were published on the solubilisation and the degradation of polysaccharides from sugar beet factory juices [11, 18, 19, 20, 21, 22, 23]. However, arabinans were still often mistaken for hemicelluloses, since hemicelluloses are extracted in much more drastic conditions than those in the sugar factory. Hemicelluloses can be defined as cell wall polysaccharides that have the capacity to bind strongly to cellulose microfibrils by H-bonds [24] and encompass xyloglucans, mannans and xylans. The common structural features of hemicelluloses are a main chain with a structural resemblance to cellulose

and either short side-chains that result in a pipe-cleaner-shaped molecule or a different sugar interpolated in the main chain, both modifications preventing further aggregation of cellulose [25, 26]. In sugarbeet, arabinans (probably by analogy with "pentosans" from cereals) are still often mistaken for hemicelluloses though they display none of their characteristics.

Few works have been published [20, 27, 28, 29] on phenolic acids associated with the soluble polysaccharides. Ferulic, p-coumaric and sinapic acids have been found in beet factory juices, but no work has been published on diferulic dimers in juices and pulps from sugar beet. The role of phenolic acids in colour formation (enzymatic browning and complexes with iron ions) during the first steps of sucrose extraction [28] is now assumed.

The overall aim of our work was to extract and characterise the pectins and hemicelluloses from sugar beet grown in Morocco and from pulps and to study the effects of these polysaccharides on beet quality and on the efficiency of sugar beet processing. The aim was also to give added value to the sugar beet pulps in Morocco which are still used as animal feeds only.

MATERIALS AND METHODS

Sampling Procedure

Samples of beet brei were taken at the receiving station of a Moroccan sugar factory, in the region of Tadla. In order to study the influence of the site crop, samples were taken from two cultivars (V_1 and V_2) grown at several sites.

Sugar beet was also sampled just before extraction in the form of beet slices. After extraction, pulps and juices were sampled. The samples of beet slices (BS), wet pulp (WP), pressed pulp (PP), raw juice (RJ) and thin juice (TJ), were taken either early (in June, beginning of the harvest season) and late (in July, middle of the harvest season) over a period of 5 and 6 days respectively, during the sugar campaign. Each day, six samples of BS, WP, PP, RJ and TJ, were taken every 2 h, immediately frozen, then mixed to obtain the mean day sample. For each period (beginning and middle of the harvest season), all mean day samples for BS, WP, PP and RJ were ground and homogenised to give the early and late samples which were treated to obtain the alcohol-insoluble solids.

Preparation of Alcohol-Insoluble Solids (AIS)

Samples of BS, WP and PP were mixed in four volumes of boiling ethanol (96%) for 15 min; after centrifugation (3,500 g, 30 min), the slurry was filtered on G3 sintered glass filter and the insoluble material was washed with aqueous ethanol (60%, v/v) until the filtrate gave a negative reaction in the phenol-sulphuric acid test [30]. The residue was dried by solvent exchange (ethanol 96% and acetone), two times each, followed by 1 night oven drying at 40 °C. The polysaccharides from raw juice were obtained by alcohol precipitation (final concentration: 77%). After centrifugation (3,500 g, 30 min), the insoluble material was washed with aqueous ethanol (70 %, v/v) and treated as the samples of BS, WP and PP.

Extractions

AIS was, as described in our previous work [31], extracted sequentially for 30 min, three times each, with distilled water (300 mL, pH 5, room temperature), 0.1 M CDTA (300 mL, pH 5, room temperature), 0.05 M HCl (300 mL, 85°C) and 0.05 M NaOH (300 mL, 4°C). After each treatment, the suspension was centrifuged for 30 min at 3,000 g, supernatants were collected and if necessary neutralised to pH 4.5 using 2 M NaOH or 1 M HCl, concentrated on a rotary evaporator, dialysed extensively against deionized water and freeze-dried. The extracts were named Water-Soluble Pectin (WSP), CDTA-Soluble Pectin (CDSP), Acid-Soluble Pectin (HSP) and Alkali-Soluble Pectin (OHSP), respectively. Pectins were also extracted from AIS of tare house samples, corresponding to different cultivars and sites, by hot water under conditions simulating factory operations: 85 °C for 10 min, 75 °C for 30 min three times, with water at pH 5.5 containing 30 mg/l NH₄Cl. After centrifugation (3,500 g, 30 min), the supernatants were pooled, concentrated under vacuum and dried.

Hemicelluloses were extracted by alkali from the residue of pectin extraction. This residue was immediately treated by sodium hydroxide (1 M NaOH then 4 M NaOH) containing 1 g/L NaBH₄, each twice for 16 h then 8 h at 4 °C. After centrifugation (5,000 g, 20 min), each supernatant was neutralised to pH 7 with 6 M hydrochloric acid, dialysed and freeze-dried. The extracts were named Hemi 1 M and Hemi 4 M.

The residue after 4 M NaOH extraction was suspended in 300 mL deionized water and the pH of the slurry was brought to 7 by careful addition of 1 M HCl. After 16 h at 4 °C under gentle stirring, the suspension was centrifuged (3,500 g, 30 min); the residue was washed (once) with an additional 300 mL deionized water. Supernatant and water washings were pooled and treated as above, giving the pH 7-soluble polysaccharides (pH7SP).

The final residue was dried by solvent exchange (ethanol 70%, v/v, ethanol 96% and acetone), three times each, followed by 1 night oven-drying at 40 °C.

Contribution of Galacturonic Acid to Colour Formation

The role of galacturonic acid was studied through a model solution with composition similar to raw factory juice. The model solution was composed of sucrose (14.5 g/100 mL), betaine (1.3 g/100 g sucrose), glucose + fructose (0.25 g/100 g sucrose), NaCl (21 mmol/100 g sucrose), KCl (23 mmol/100 g sucrose), glutamine and aspartic acid (5.65 mmol/100 g de sucrose, each). Galacturonic acid was added to the model solution to obtain different concentrations (0, 6, 12, 24, 30 and 50 mg/100 mL).

The pH of 100 mL of the model solutions was adjusted to 9 before evaporation under atmospheric conditions during 50 min; the colour was then measured by ICUMSA method at 420 nm; three repetitions were made.

Ion-Exchange Chromatography

Chromatography on a column (10.5 x 3.2 cm) of DEAE Sepharose CL-6B (Pharmacia), followed equilibration with 0.05 M ammonium succinate buffer (pH 4.8). After loading 50-100 mg of the extract, the column was washed over one hour with equilibrating buffer 0.05 M at 0.9 mL/min, eluted by 300 mL of a linear gradient (0.05 to 2 M of ammonium succinate) and then washed by 2 M ammonium succinate for 45 min. Fractions of 3.5 mL were collected and analysed for their galacturonic acid and neutral sugars contents. For the elution of hemicelluloses (Hemi 1 M and Hemi 4 M extracts), after washing with 2 M ammonium succinate, the column was further washed with 0.5 M sodium hydroxide. Appropriate fractions were pooled, dialysed and freeze dried.

Analytical Methods

The dry matter was been determined by drying at 120°C for 8 h. Uronic acids in AIS and extracts were determined by the automated m-hydroxybiphenyl method [32], and expressed as anhydrogalacturonic acid. Total neutral sugars in pectins (expressed as "anhydroarabinose") and in hemicelluloses (expressed as "anhydroglucose") were determined with the automated orcinol method [33]. Corrections were made for interference by uronic acids in the neutral sugars assay. Pectins in raw and thin juices were determined by the m-hydroxybiphenyl method after methanol precipitation and centrifugation and expressed as anhydrogalacturonic acid [34].

Proteins were measured by the Kjeldahl method (protein conversion factor: 6.25) for the AIR, and by the Bradford method in the extracts. Methyl ester groups were determined by HPLC, after alkaline deesterification. From these contents and the contents in galacturonic acid, the degree of methylation (DM) was calculated, and expressed as moles of ester per 100 moles of galacturonic acid.

Individual neutral sugars contents were determined by gas liquid chromatography: pectins and hemicelluloses were hydrolysed in 1 M sulfuric acid at 100 °C for 6 h; the sugars were reduced by sodium borohydride, acetylated and their alditol acetates analysed by GLC [35]. For the AIR and the extraction residue, the duration of hydrolysis was 3 h after pre-treatment by 13 M sulfuric acid for 1 h at 25 °C [35]. Monomers and dimers of ferulic acids were measured in the AIS of PP by HPLC [36].

RESULTS AND DISCUSSION

Yields and Composition of the AIS

Yield and composition of AIS in sliced beet were typical of sugar beet AIS [8, 10, 11]: the yield in AIS was 5 % and 6 % of fresh beet respectively for the Early and Late samples (about 23% of beet dry matter, **Table 1**). The neutral sugars were represented mostly by glucose, arabinose, galactose, xylose and mannose, as usual in sugar beet. Protein concentration (90-92 mg/g) was typical of sugar beet AIS [8]. Few differences were observed between the early and late samples. Concerning the pressed pulp, yield and composition of AIS were different depending probably on the factory extraction conditions (**Table 1**). Before pressing, AIS represented 62.9 and 61.7 %

of Early and Late dry wet pulps, respectively. After pulp pressing, AIS content was 94.1 and 82.8 % of Early and Late dry pressed pulps, respectively (about 24.0 % of fresh matter).

Table 1. Yields (% dry initial material) and compositions in sugars (mg/g dry AIS) and proteins (mg/g dry AIS) of AIS from sliced beet, wet pulps and pressed pulps at two stages of the campaign, early (E) and Late (L).

	Yield (% dry matter)	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	AUA	Proteins
Sliced beet										
E	24.5	15	2	143	19	13	43	204	173	90
L	21.8	14	1	144	18	12	43	194	182	92
Wet pulps										
E	62.9	16	2	156	17	12	44	214	168	84
L	61.7	13	1	153	21	11	37	195	190	82
Pressed pulps										
E	94.1	14	2	164	23	12	40	211	171	82
L	82.8	12	2	129	33	10	40	205	170	75

Yields of Extraction of Polysaccharides Under Laboratory Conditions

All values are presented on AIS dry weight basis (**Tables 2 and 3**). Total pectins represented 32.6, 35.1 and 39.1 % dry AIS respectively for BS, WP and PP from Early samples. For the Late samples, the yields were 32, 34.4 and 36.4 % dry AIS respectively for SB, WP and PP. The highest extraction yields were obtained for the alkali and dilute acid treatments (**Tables 2 and 3**) while only limited material was extracted by water (2.0-2.2 % for beet slices and 1.3-1.6 % for the pulps) and CDTA. (0.4 –0.8 % for beet slices and 0.4 - 0.7 % for the pulps) These values are in the range of the values found in the literature [8, 10, 11]. Concerning the pectins extracted by hot water, marked differences in yield (3.1 – 5.0 % AIS) were observed between samples of tare house corresponding to two cultivars. Simulating factory conditions gave a higher yield of extraction than cold water.

Yields for hemicelluloses after extraction with 1 M NaOH from beet slices were 12 % and 13 % of AIS, respectively, for Early and Late beet slices, and after extraction with 4 M NaOH 5 % and 6 %, respectively. These values are relatively high compared to other plants. Thus, for apple, the total yield was 15 % of AIS treated by CDTA [37]. For *Salicornia ramosissima*, also a Chenopodiaceae, using 4 M NaOH, the yield obtained was 9 % [38]. As in our previous work [22] few pectins were extracted at pH 7 (2.4 and 2.2 % AIS, respectively for Early and Late samples) as described by Renard et al. [39] on other material than sugar beet. From AIS of pressed pulps, yields of extraction of hemicelluloses were 6.1 % and 5.5 % of AIS, with 1M and 4M NaOH, respectively.

Table 2. Yields (% dry AIS) and compositions in sugars (mg/g dry extract) of pectins extracted from AIS of the early beet slices, wet pulps and pressed pulps, sequentially with water, 0.1 M CDTA, 0.05 M HCl, 0.05 M NaOH and at neutralization (pH7).

	Yield (% dry AIS)	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	AUA
Sliced beet									
WSP	2.2	12	0	99	7	22	112	29	282
CDSP	0.4	10	0	55	6	9	44	7	371
HSP	12.2	13	0	264	1	0	27	3	427
OHSP	15.4	33	0	134	2	0	59	0	371
PH7SP	2.4	64	0	138	7	0	113	0	109
Wet pulps									
WSP	1.3	12	0	105	5	14	81	22	386
CDSP	0.4	9	0	68	6	10	56	18	385
HSP	14.8	14	0	287	0	0	32	0	347
OHSP	18.6	35	0	159	3	0	65	0	563
Pressed pulps									
WSP	1.6	15	1	131	9	11	83	17	376
CDSP	0.7	8	0	70	4	9	50	9	322
HSP	14.0	17	1	349	0	0	36	1	315
OHSP	22.8	41	1	157	2	0	75	0	390

Table 3. Yields (% dry AIS) and compositions in sugars (mg/g dry extract) of pectins extracted from AIS of the late beet slices, wet pulps and pressed pulps, sequentially with water, 0.1 M CDTA, 0.05 M HCl, 0.05 M NaOH and at neutralization (pH7).

	Yield (% dry AIS)	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	AUA
Sliced beet									
WSP	2.0	13	0	82	6	17	98	22	290
CDSP	0.8	11	0	44	4	6	33	7	388
HSP	11.6	14	0	289	0	0	28	0	376
OHSP	15.4	28	0	125	1	0	49	0	546
PH7SP	2.2	60	0	152	8	0	110	0	21
Wet pulps									
WSP	1.3	17	0	134	9	16	111	20	352
CDSP	0.5	9	0	67	6	10	57	11	355
HSP	12.6	11	1	310	0	0	25	1	254
OHSP	20.0	37	1	215	2	0	68	1	526
Pressed pulps									
WSP	1.4	13	0	105	9	9	78	19	326
CDSP	0.5	9	0	68	6	10	53	12	392
HSP	11.8	14	0	347	0	0	28	0	326
OHSP	22.7	35	1	123	2	0	62	1	433

The final residues represented 46 % and 43 % of AIS for Early and Late samples, respectively. Their compositions indicated that a high concentration of glucose (67 - 68 % of total moles of neutral sugars) was present; presumably this was of cellulosic origin, a small amount of mannose and still 153 mg/g of uronic acids.

Composition of Pectin and Hemicellulose Extracts

The compositions of pectins extracts from BS, WP and PP are given in **Tables 2** and **3**, respectively for Early and Late samples. Extracts obtained by acid and alkali treatments were particularly rich in uronic acids (315-563 mg/g), arabinose (134-349 mg/g) and rhamnose (13-41 mg/g) while the pectins solubilised by water contained significant amounts xylose (7-9 mg/g), mannose (11-22 mg/g) and galactose (81-112 mg/g). The analysis of the polysaccharides extracted at pH 7 showed a composition characteristic of pectins with the presence of arabinose, galactose and rhamnose with a rather low concentration of uronic acid. They differed from the pectins extracted at the beginning of the sequence (WSP) by higher rhamnose and galactose concentrations (**Tables 2** and **3**).

Comparison between extracts from sliced beets, wet pulps and pressed pulps showed that some sugars in sliced beet (mannose, glucose) are extracted during the treatment with water but not by NaOH (**Figure 1**). For the fractions extracted by CDTA, the sugar contents were low because the persistence of high amounts of CDTA in spite of extensive dialysis. The high arabinose contents may be due to the use of dialysis instead of copper [40] or alcohol precipitation [8, 40, 10] to obtain the polysaccharides, as arabinans are known to be soluble in ethanol in aqueous solutions.

The hemicellulose extracts were composed mostly of arabinose (**Figure 2**), which had not been totally extracted during extraction steps to remove pectins, xylose and glucose, with relatively high concentrations of fucose and mannose. For comparison, alkali extracts from *S. ramosissima* are rich in xylose and glucose but not in arabinose [38]. The compositions of Hemi 1 M and Hemi 4 M extracts were very different: the Hemi 1 M extracts were rich in neutral sugars and still contained a notable concentration of uronic acids, measured as anhydrogalacturonic acid (50 – 65 mg/g extract), while the Hemi 4 M extracts were poor in uronic acids, xylose and arabinose (**Figure 2**).

The hemicelluloses extracts from BS were rich in arabinose while the extracts from PP were rich in xylose, mannose and glucose which are typical sugars of hemicelluloses (**Figure 2**). Those differences would suggest that neutral chains of arabinans, which are associated with hemicellulose are extracted during the first steps of the process. The typical sugars of hemicelluloses (xylose, mannose and glucose) are more concentrated in PP than in BS. In a previous work [41], we have shown the presence of heteroxylans in addition to xyloglucans in hemicelluloses extracted from BS. From composition of sugar beet pulp extracts, Wen et al. [17] have inferred presence of xylans and mannans, and [40] has identified xyloglucans and glucomannans.

Chromatography on DEAE Sepharose CL-6B of the Pectin and Hemicellulose Extracts

The use of ammonium succinate instead of sodium succinate or acetate allowed higher recoveries [31]. For the pectins extracted by NaOH from AIS, nearly the same elution profile were obtained both for BS and PP (**Figures 3 and 4**, respectively). These profiles contained both retained and non-retained fractions in varying proportions. The non-retained populations were mostly composed of neutral sugars while the retained fractions were rich in anhydrogalacturonic acid and poor in neutral sugars.

The separation of hemicelluloses extracted from BS and PP on DEAE Sepharose CL-6 B gave different elution profiles (**Figures 5 and 6**) confirming that some fractions of arabinans associated to hemicelluloses could be extracted during the first step in the sugar beet processing.

Extraction and Degradation of Pectins Under Sugar Factory Conditions

In raw juice, the yield of alcohol insoluble polysaccharide represented only 2.2 and 1.9 mg/g dry matter, respectively for Early and late samples. However, these values are higher than those given by Vogel & Schiweck [18] (1.3 mg/g dry matter). Polysaccharides in raw juice showed a composition characteristic of pectins (**Figure 7**): Galacturonic acid is the most important sugar (58-65 % weight of total sugars) in agreement with De Bruijn et al. [19]. Similar composition was observed for the samples of tare house after extraction under factory simulating conditions (**Figure 8**). The other sugars of polysaccharides from raw juice were represented by arabinose and galactose. However, big variation in composition of neutral sugars of AIS RJ can be observed (**Figure 9**) depending probably on conditions of extraction (pH and temperature). Some samples were rich in glucose which was present as neutral polymers, probably dextrans coming from microbial degradation of beet after harvesting as shown by many studies [23, 42]. It is usual under hot climate that a degradation of beet quality [1, 2], as synthesis of microbial polysaccharides [23, 42], occurs during the harvest season. This situation highly influences the sucrose yield in the factory.

The concentration of pectins in the raw juice (400-830 mg/100 g sucrose) varied along the two periods of campaign (**Figure 10**) depending probably on the beet quality as well as on the extraction parameters [43]. Pectins still remained in thin juice after the purification step where hydrolysis and β -elimination reactions occur at high pH and temperature; the average concentration was 14 mg AUA/100 g juice (85 mg/100 g sucrose) and 16 mg AUA/100 g juice (130 mg/100 g sucrose), respectively for the period 1 and the period 2 (**Figure 10**). These different concentrations should be correlated with the different purification conditions in the two periods [43]. The pectins in thin juice were polymeric since the analysis was done on the alcohol precipitate. Considering that few pectins were present as oligomers in raw juice, the results suggested that 13% and 26% of the pectins from raw juice, during period 1 and period 2, respectively, still remained in polymer form at the end of the purification step and will go through the evaporation process and reach molasses as shown by many studies [18, 19, 20]. Chromatography of the alcohol precipitate from thin juice showed almost a non-retained peak [43]. The arabinose represented 38 mol %.

The dry matter in the pressed pulps, which is an important parameter in the extraction step is related to the level of polysaccharides extracted (**Figure 11**). More the polysaccharides extracted in the raw juice, lower their concentrations in the wet pulps and therefore, weaker the linkage with calcium sulphate added to increase pressing and dry matter.

Role of Galacturonic Acid in Colour Formation

The presence of monomers of galacturonic acid in the model solution significantly increased the colour formation when the solution was evaporated (**Figure 12**). Thus, the hydrolysis of pectins during purification in the factory should give monomers of galacturonic acid and therefore affect negatively the processing performances through colour formation and ionic balance.

Adding Value to the Pressed Pulp

AIS represented an average of 91% of dry matter in the pressed pulp. Compared with AIS of the pressed pulp from temperate climate, AIS of Moroccan sugar beet pulp was very rich in xylose, but had lower arabinose and uronic acid contents (**Figure 13**). Concerning proteins, the AIS contents were 78.6 mg/g while for Van der Poel, et al. [20] and Clarke [21], protein concentrations were 114 and 54-109 mg/g, respectively. Degrees of methylation of PSH from AIS PP were similar to published data (66%).

The amount of monomers and dimers of ferulic acid in pressed pulps could also be used to give a high technological value to this by product [44]. In fact, under temperature and pH of the sucrose extraction step, pectins were solubilised in the raw juice factory [43] with their associated phenolic acids. However, since the pH (5.5 – 6.5) and temperature (70-75 °C) conditions in the diffuser are not enough for extraction of phenolic acids associated with cell wall polysaccharides, the major part of these compounds remained in the pulp. In agreement with published data [8, 44, 45, 46] ferulic acid represented 7.2 and 7.6 mg/g AIS, for early and late PP, respectively (6.6 and 6.8 mg/g dry matter of PP). It was the major (90.9 – 94.1%) phenolic acid present in sugar beet pulp. Total diferulic acid represented 0.4 mg/g AIS of pressed pulp in agreement with the results of Micard et al. [46]. As in beet slices, 8-O-4' was the major dimer with little difference between the two periods (59% of total diferulic acid on average), while, for Micard et al. [47], the most abundant diferulic dimer in the PP was the 8-5' dimer.

All these characteristics of AIS PP could be used to give an added value to the PP in Morocco starting by titration of AIS and pectins extracts to determine the number of anionic sites (**Figure 13**). These sites can be tested for decolorization of thin juice (**Table 4**). Other properties should be tested.

Table 4. ICUMSA colour of thin juice before and after mixture with AIS

ICUMSA Color Before	ICUMSA Color After	% decolorization
5450	5000	16.51
5478	5012	17.04
5478	4900	21.14
6213	5506	22.76
6213	5568	20.76
Average:		16.64 %

CONCLUSION

Polysaccharides in sugar beet grown under Mediterranean climate have nearly the same composition comparatively to the beet slices from temperate climate. During beet processing, the polysaccharides extracted have a composition characteristic of pectins; however, some arabinans associated to hemicelluloses could be extracted. The effects of polysaccharides on beet processing depend on the extraction and purification conditions; some effects, especially on colour formation were shown. Although the few amount of polysaccharides extractable under factory conditions, polysaccharides can be considered as non conventional elements of beet quality.

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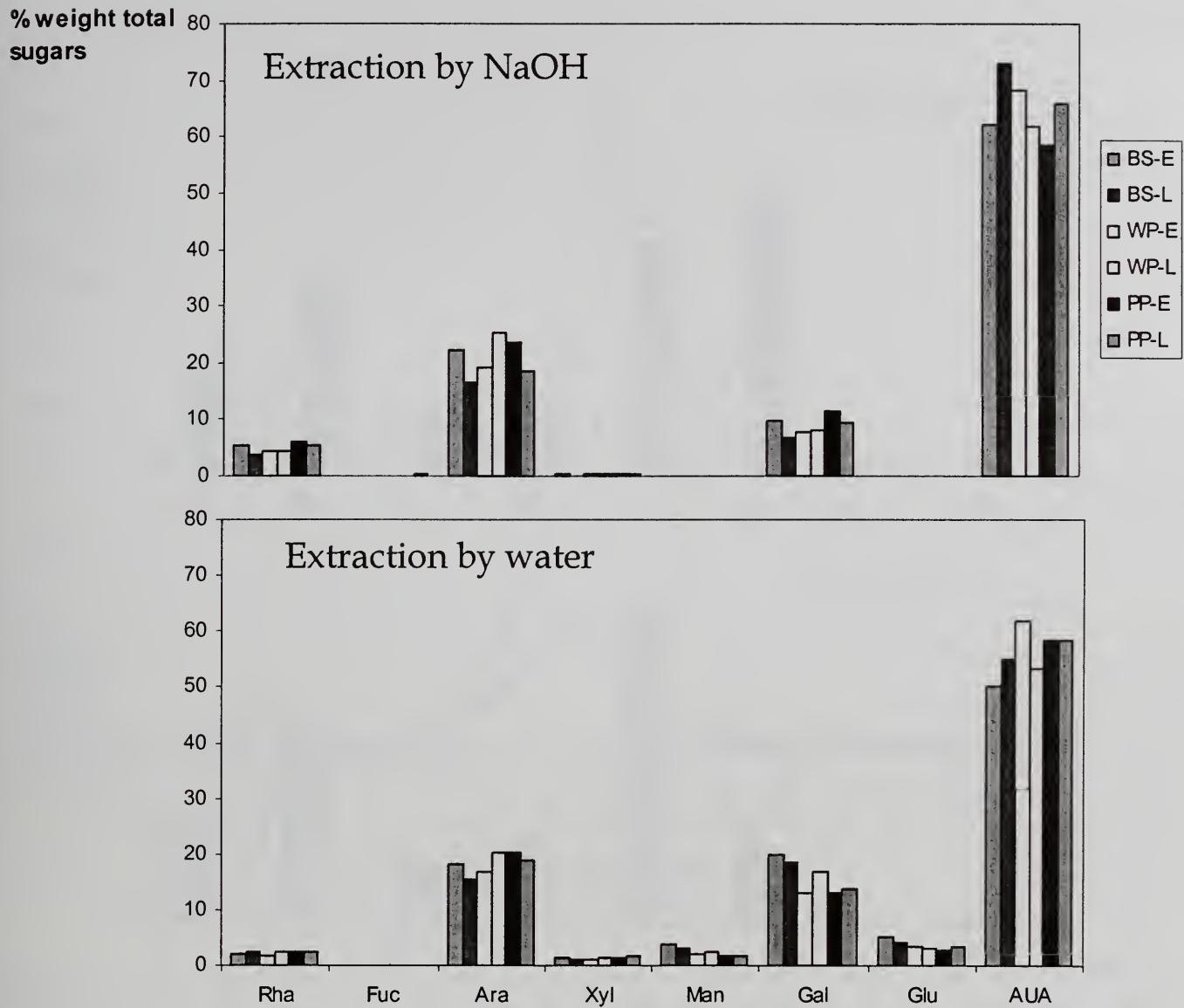


Figure 1. Variation in the composition of pectins at different steps of beet processing.

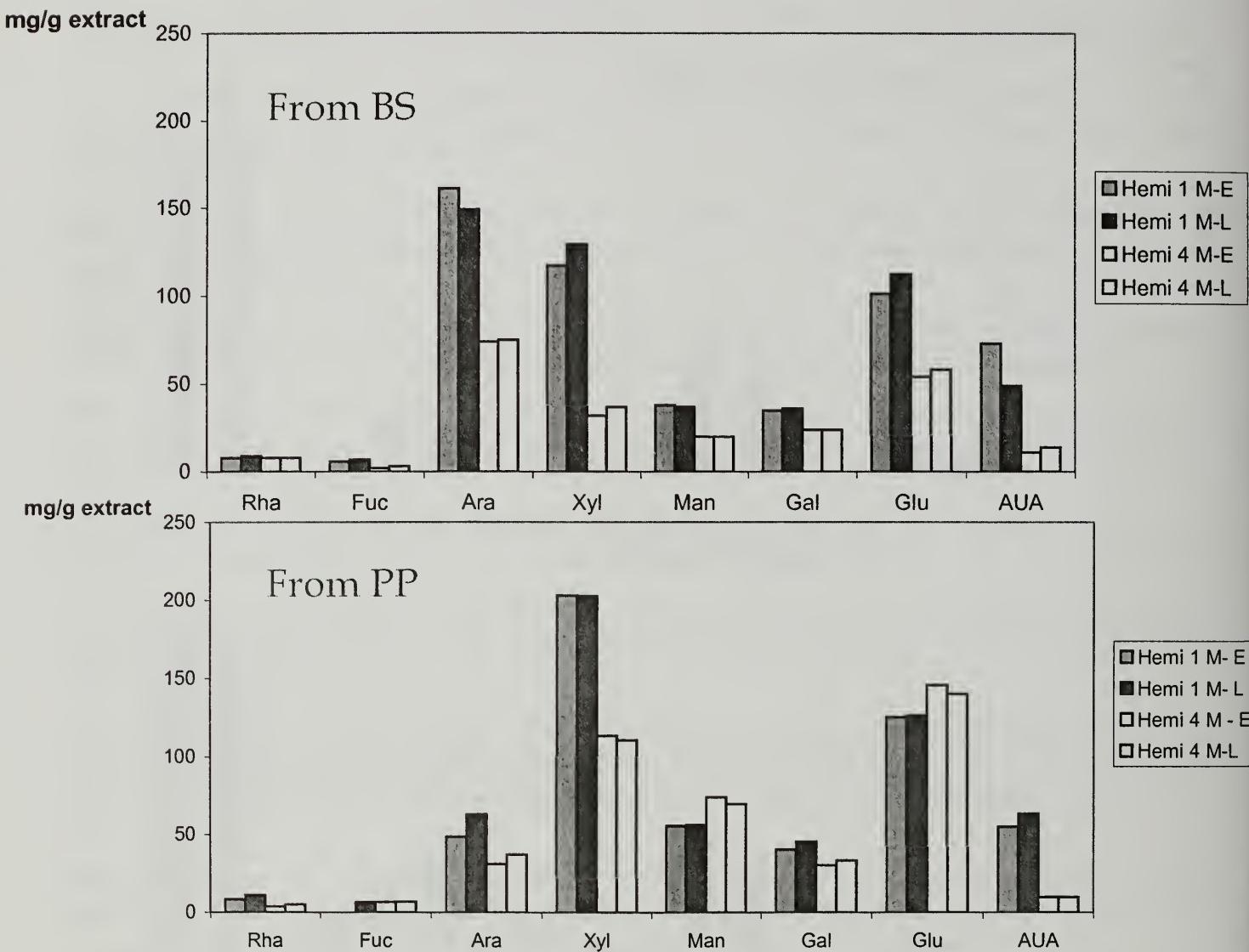


Figure 2. Compositions (in mg/g extract) of hemicelluloses extracted from AIS of Early (E) and Late (L) Beet slices and pressed pulps samples with 1 M NaOH (Hemi 1 M) and 4 M NaOH (Hemi 4M).

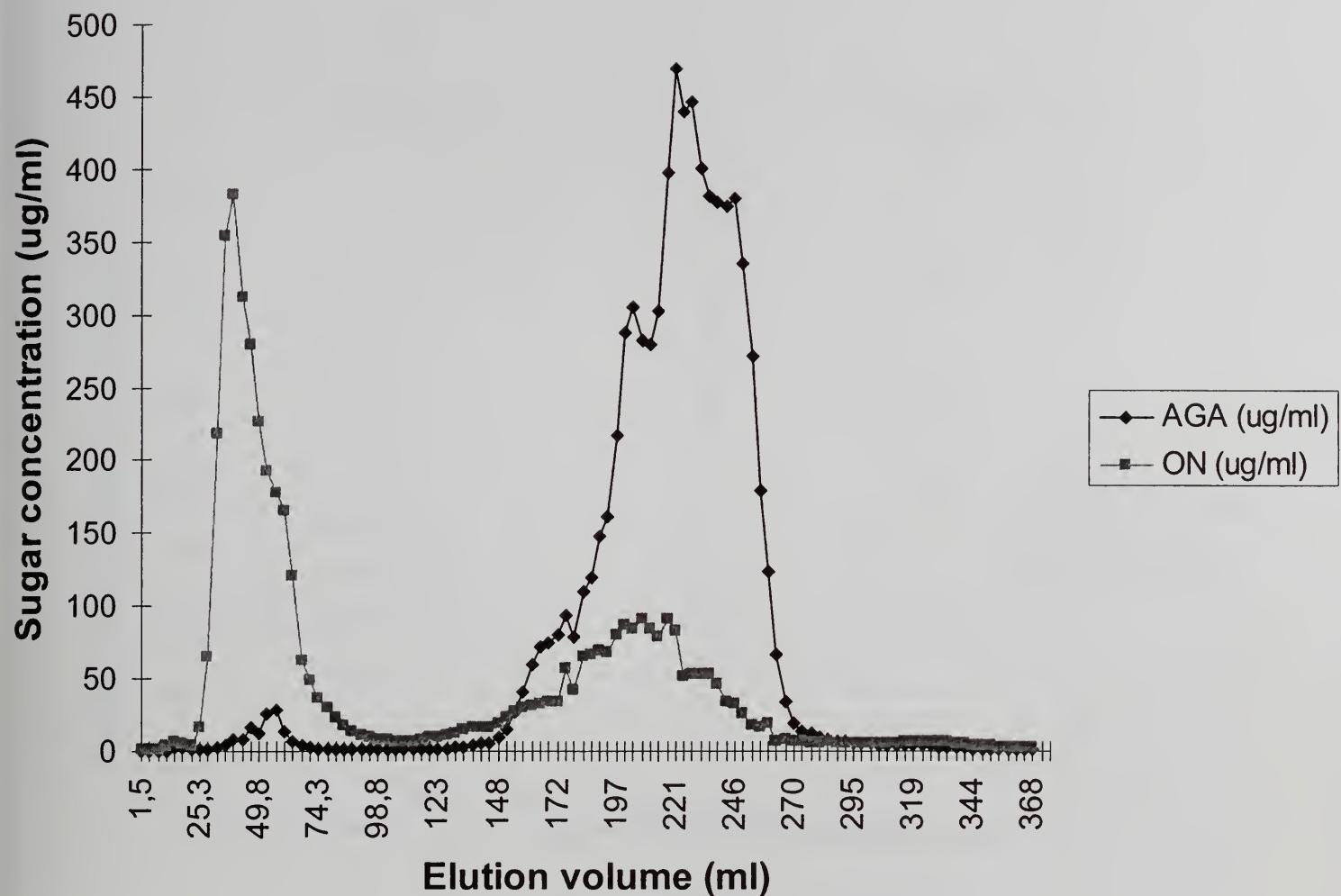


Figure 3. Elution profiles of pectic fractions on DEAE Sepharose CL-6B (eluted by ammonium succinate buffer pH 4.8), after extraction from AIS beet slices with NaOH (PSOH). AGA: Anhydro-Galacturonic acid, ON: neutral sugars

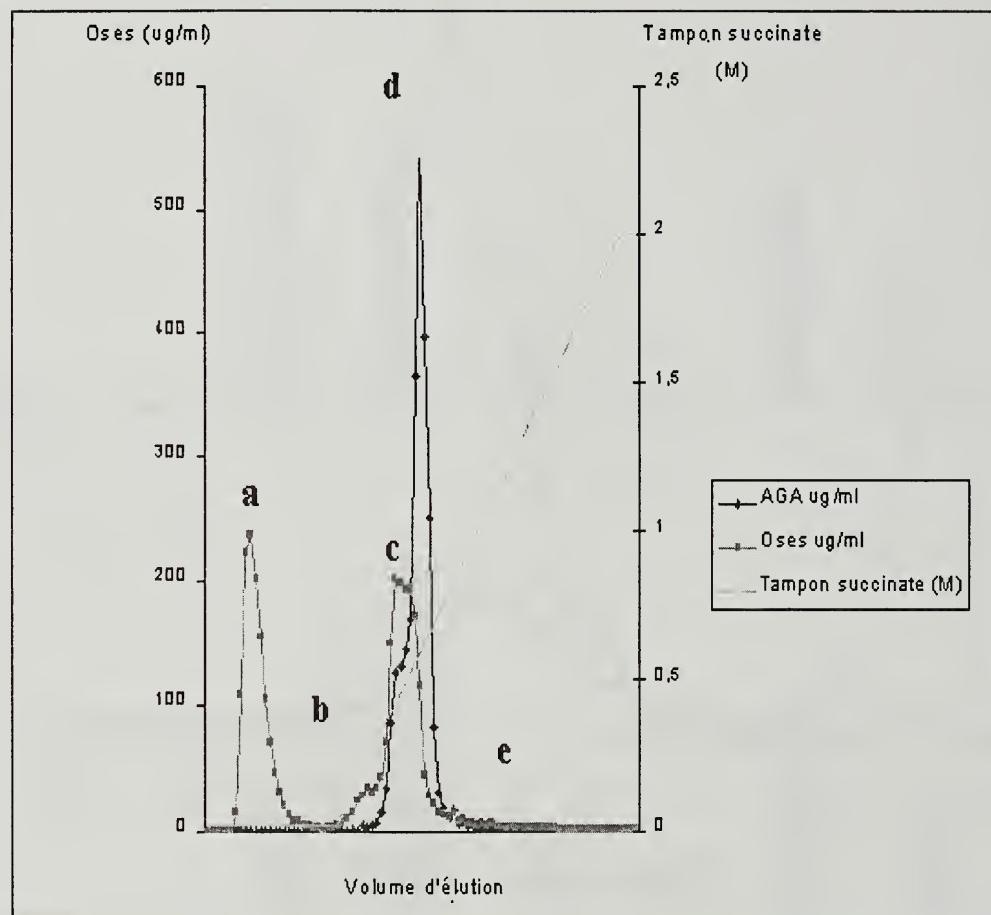


Figure 4. Elution profiles of pectic fractions on DEAE Sepharose CL-6B (eluted by ammonium succinate buffer pH 4.8), after extraction from AIS pressed pulps with NaOH (PSOH). AGA: Anhydro-Galacturonic acid.

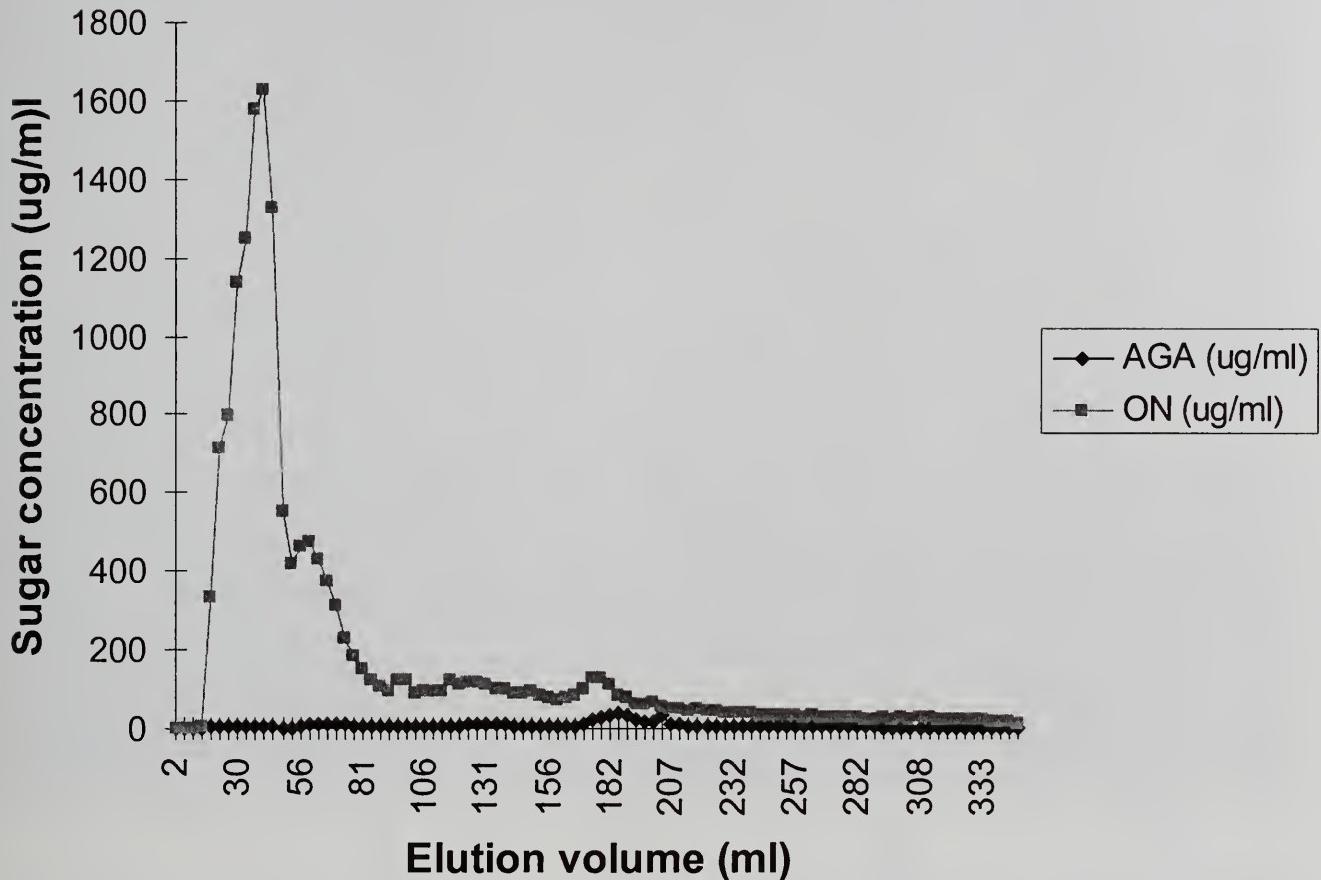


Figure 5. Elution profiles of hemicelluloses fractions on DEAE Sepharose CL-6B (eluted by ammonium succinate buffer pH 4.8), after extraction from AIS beet slices with 1 M NaOH (Hemi 1 M). AGA: Anhydro-Galacturonic acid, ON: neutral sugars.

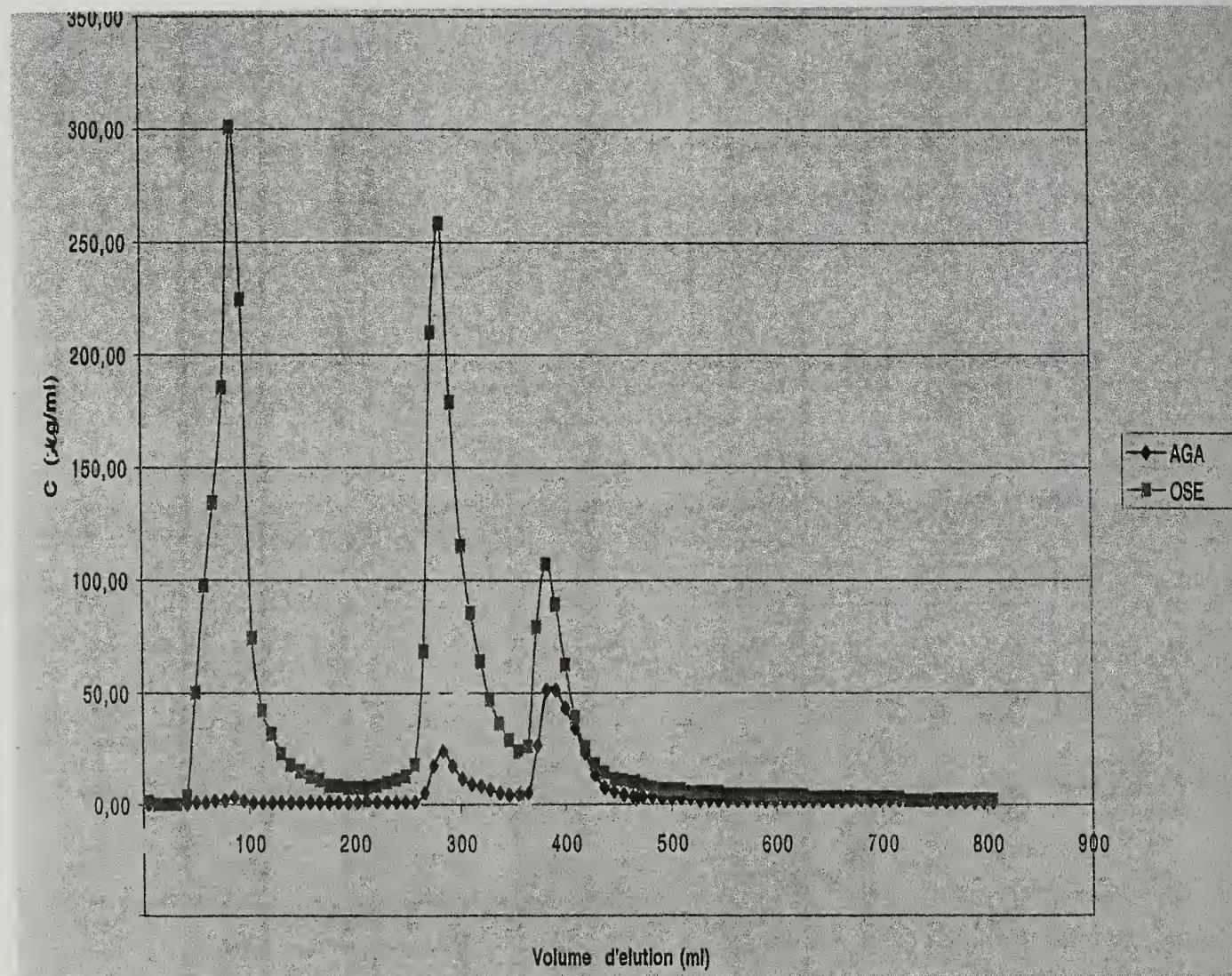


Figure 6. Elution profiles of hemicelluloses fractions on DEAE Sepharose CL-6B (eluted by ammonium succinate buffer pH 4.8), after extraction from AIS pressed pulps with 1 M NaOH (Hemi 1 M). AGA: Anhydro-Galacturonic acid.

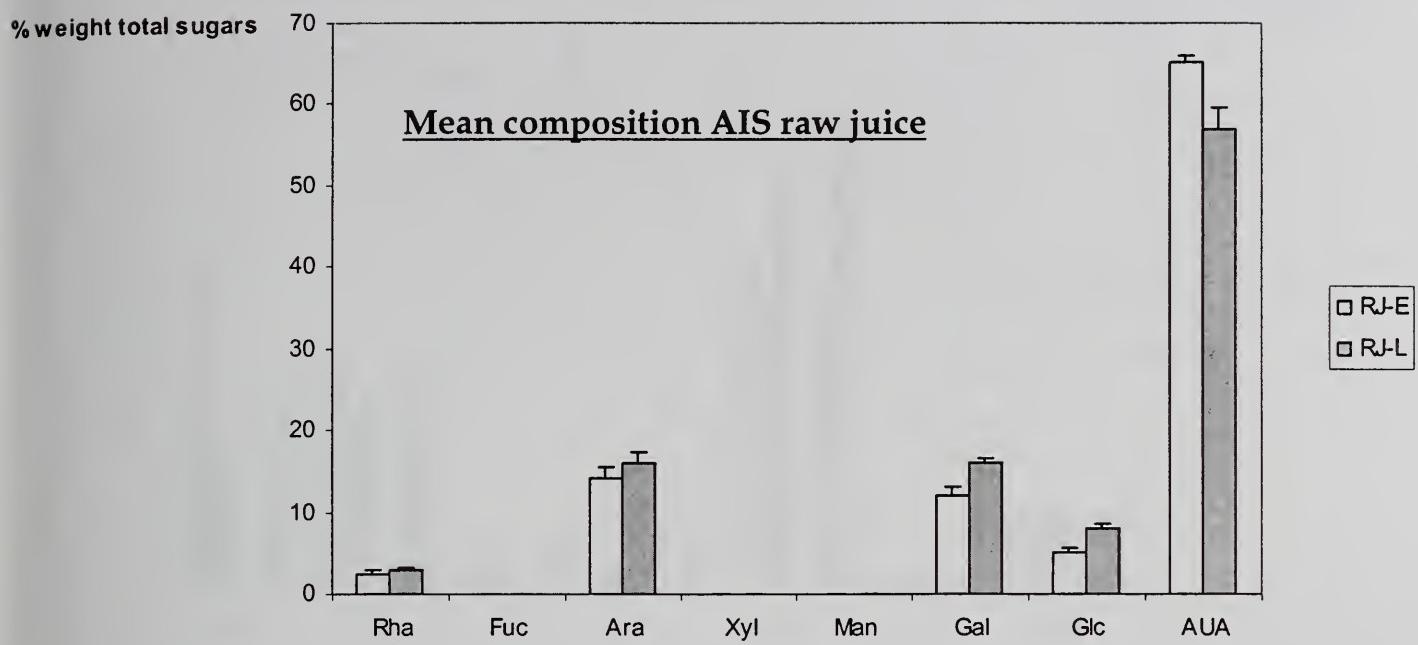


Figure 7. Mean composition in sugars (% weight of total sugars) of AIS raw juice early (RJ-E) and late (RJ-L)

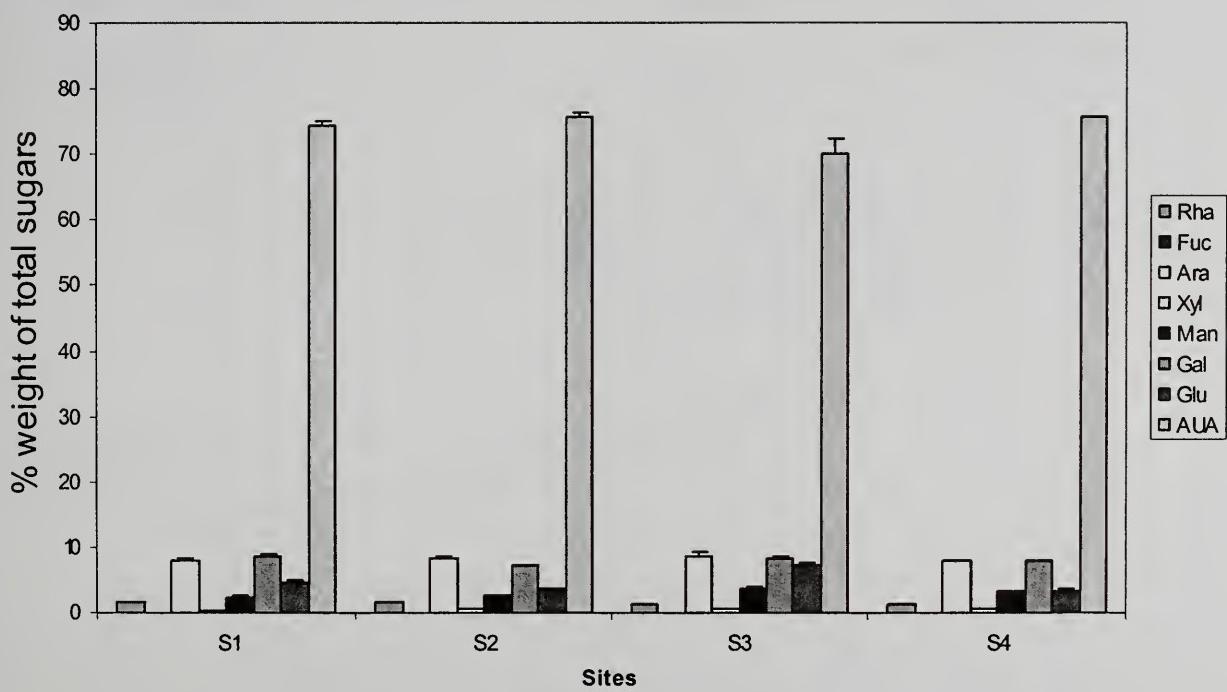


Figure 8. Variation in composition in sugars (% weight of total sugars) of pectins extracted, under simulating factory conditions, from beet samples of one cultivar coming from 4 sites (S1, S2, S3 and S4).

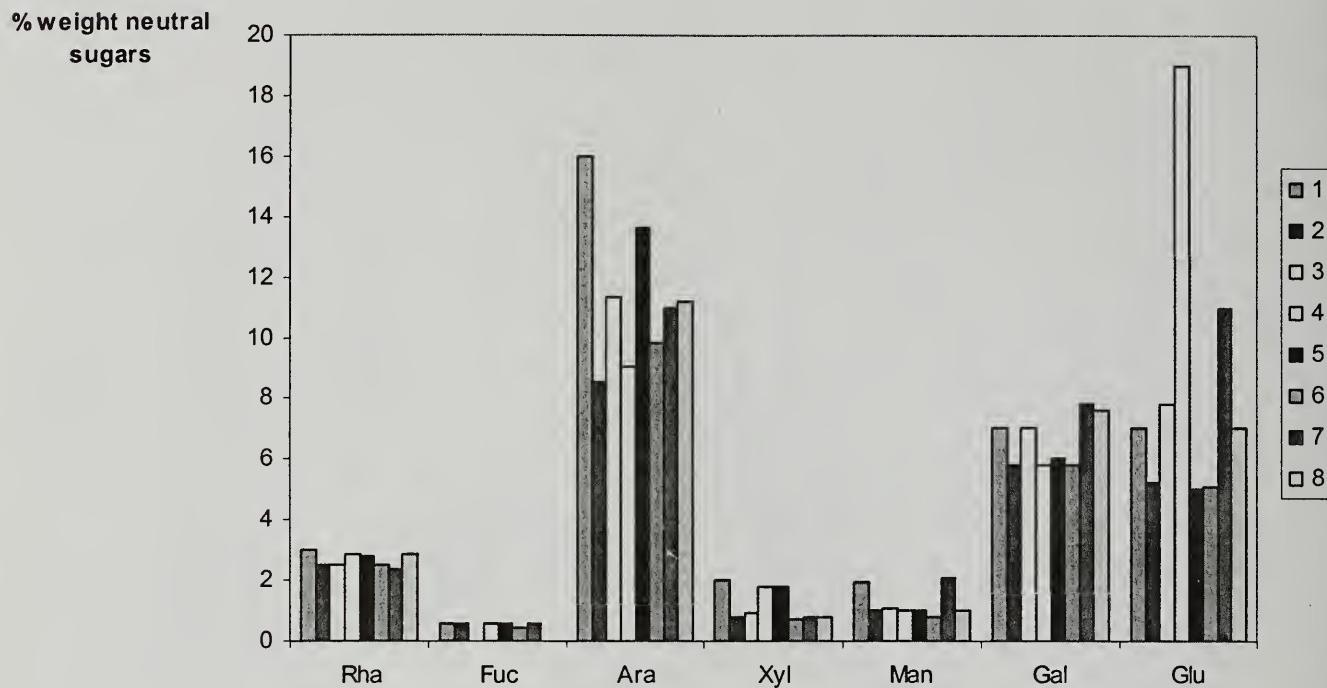


Figure 9. Variation in composition in sugars (% weight of neutral sugars) of AIS of 8 raw juices samples)

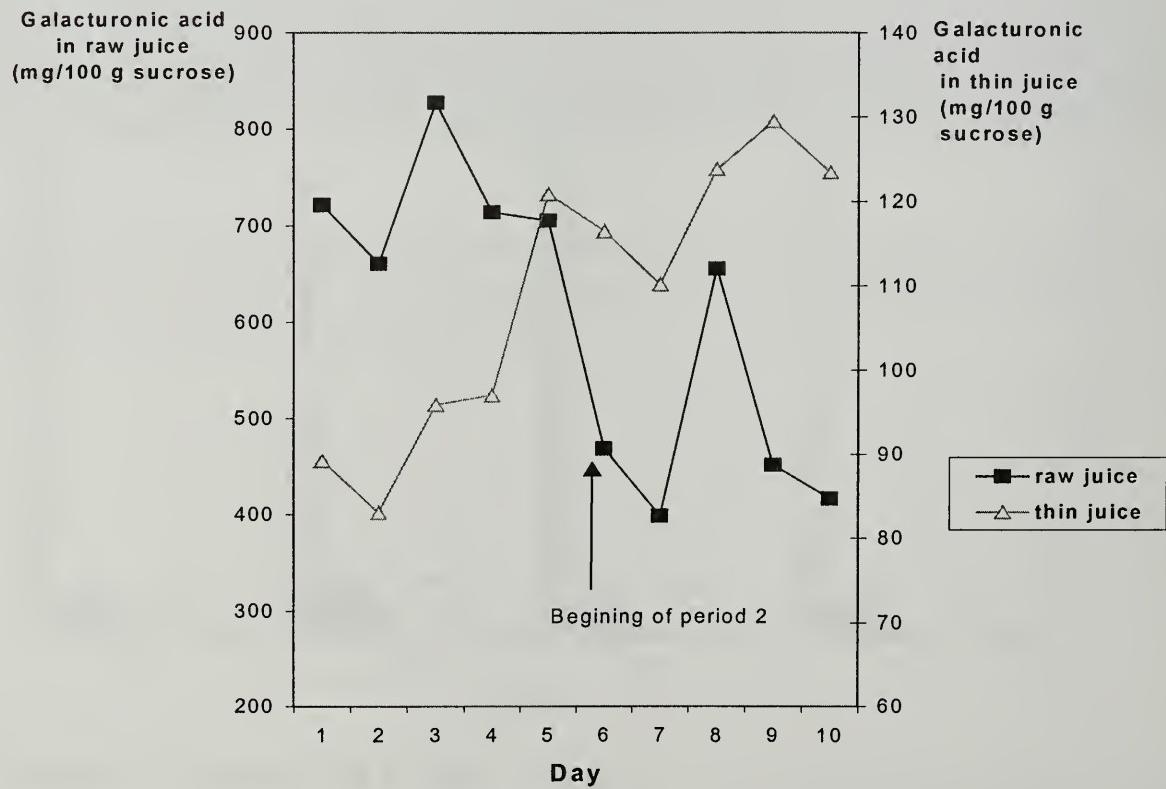


Figure 10. Variation of the concentration of galacturonic acid (mg/100 g sucrose) in raw juice and thin juice from factory during the two periods of sampling.

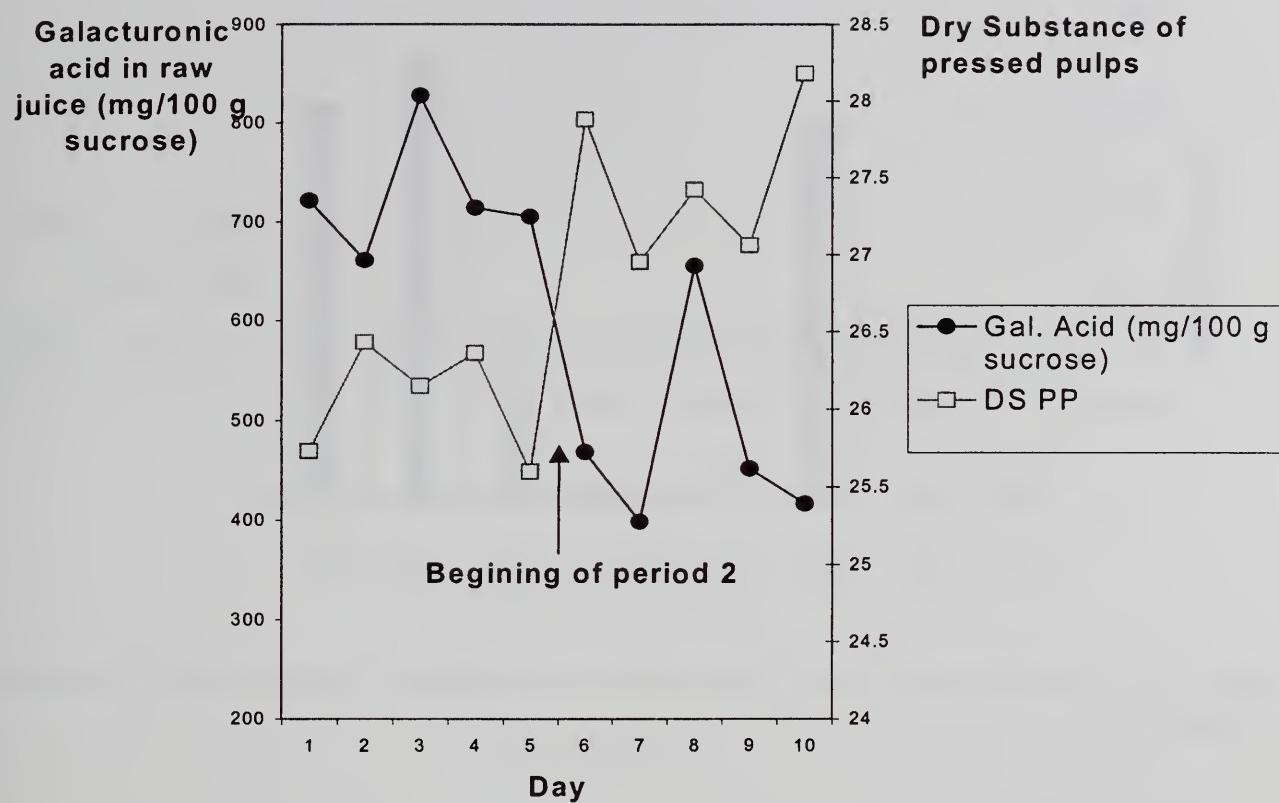


Figure11. Relation between the concentration of pectins in raw juice (expressed as mg galacturonic acid/ 100 g juice) and the dry substance in the pressed pulps (%).

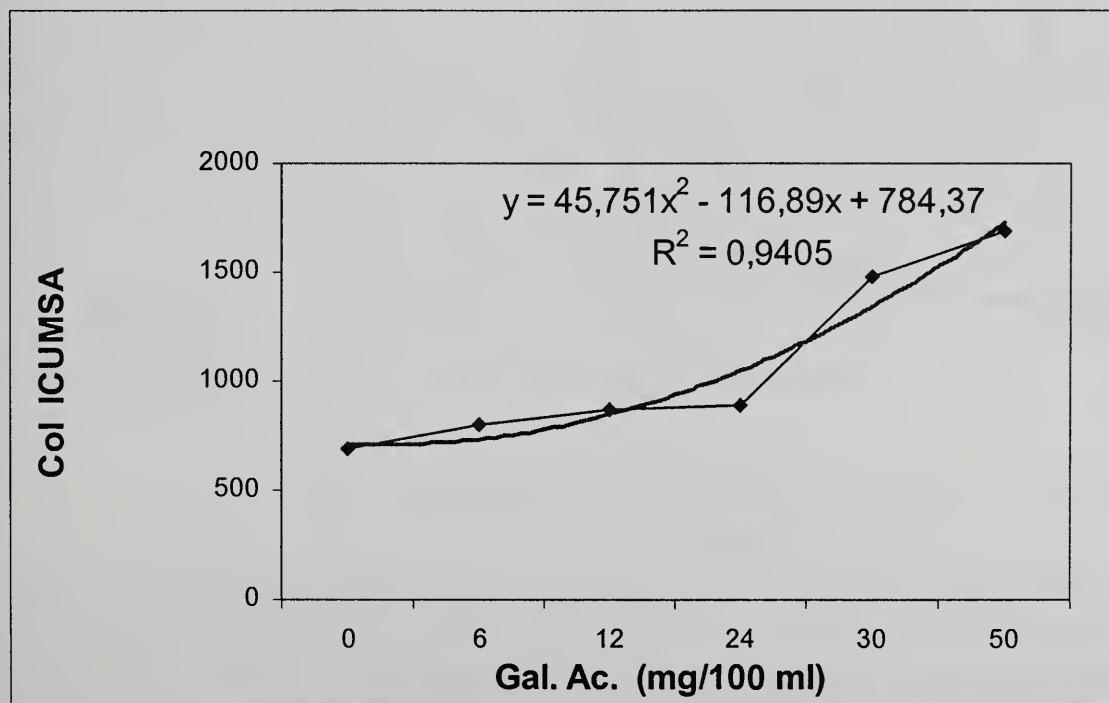


Figure12. Variation of the colour intensity in the model solution when the concentration of galacturonic acid increase.

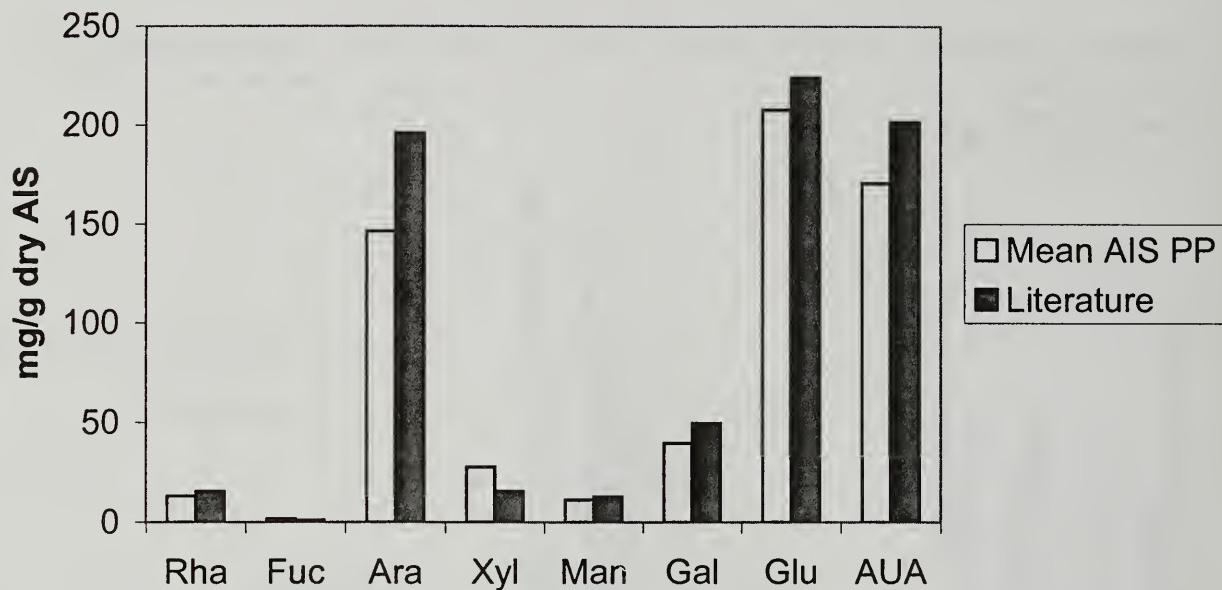


Figure 13. Comparison of mean composition of Moroccan AIS pressed pulp with data from literature.

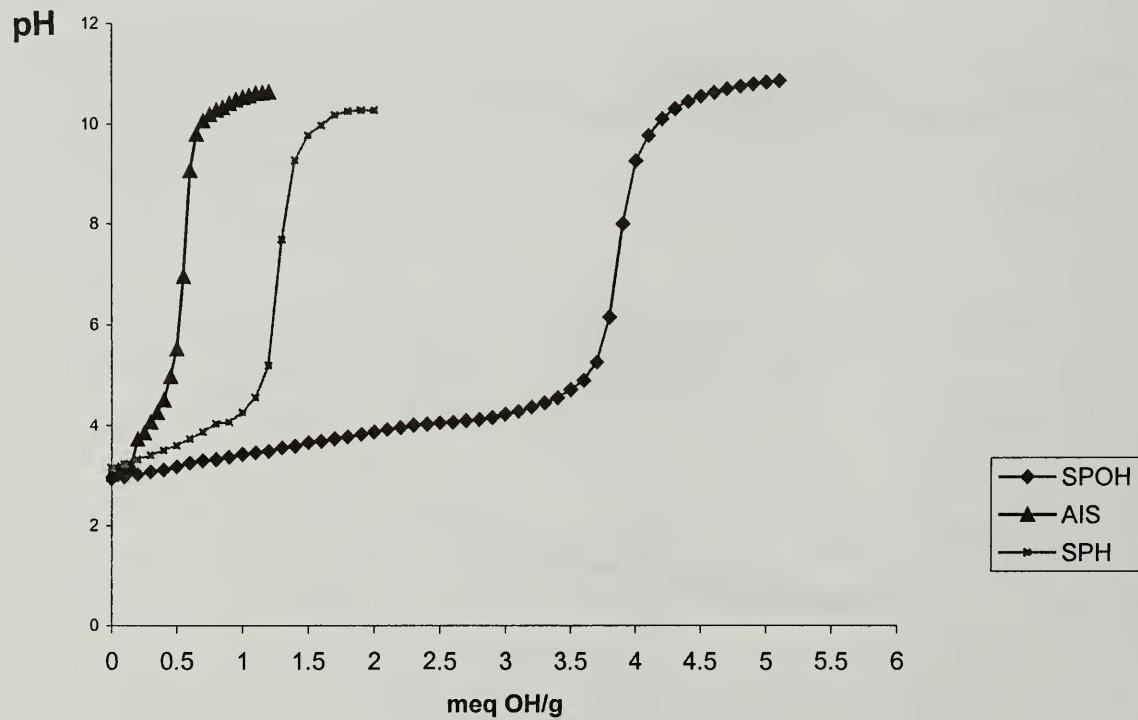


Figure 14. Titration of AIS and pectin extracts from pressed pulps.

THE EFFECT OF OZONE, HYDROGEN PEROXIDE AND SULFITE ON CANE AND BEET MACROMOLECULES

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ABSTRACT

Ozone, hydrogen peroxide and sulfite are powerful agents with activity against sugar colorant. All have been used at one time or another in sugar processing, with sulfite by far the most commonly used. Sulfitation, used in the production of plantation white sugar and in the production of white beet sugar, results in less color transfer into the crystal than the normal raw cane sugar process, which uses no sulfitation. Thus, the effect of sulfite on colorants and on color transfer is of great interest. If other decolorizing agents, such as ozone and hydrogen peroxide, are used as alternatives to sulfite, will they have the same beneficial effect on color transfer? Preliminary studies reported here show that ozone and hydrogen peroxide have little effect on the polysaccharide content of cane juice, but sulfite removed some 2 to 12 percent. Gel permeation chromatography showed that hydrogen peroxide and ozone had little effect on colorants in beet thick juice. Ozone had little effect on cane juice colorants, but hydrogen peroxide significantly diminished a major colorant peak in cane juice. Sulfitation removed color and polysaccharide in laboratory clarification of cane juice and also had significant but complicated effects on the GPC colorant profiles of both cane and beet sugar, diminishing some peaks and shifting some peaks to a lower molecular weight range.

INTRODUCTION

The production of good quality white sugar, whether it is from the cane or the beet plant, has always been about "getting the color out." A wide array of schemes, chemical aids and processes has been employed to do this. Among the chemicals used to assist in purification, almost always in some combination, are lime, sulfite, anionic flocculents, phosphoric acid and CO₂. There has been some speculation about the potential for using chlorites and cationic coagulants and a lot of interest in ozone and hydrogen peroxide. Sulfite, ozone, hydrogen peroxide and chlorite are sometimes referred to as bleaching agents. Sulfite and chlorite accomplish their bleaching action by reduction, while

ozone and hydrogen peroxide accomplish it by oxidation. These compounds could, as a class, be loosely referred to as "oxygen active." The oxygen actives do not remove color, but rather change it to colorless or less colored molecules. There is always the potential that the changed/bleached color will revert back to its colored form, so the oxygen-modified colorants still should be removed for best effect. In the beet industry, most of the color is removed by crystallization, following extensive clarification using lime, carbonation and sulfitation. Cane colorants are less removed by crystallization, so processes involving physical removal of colorants are employed, and include, alone or in combination, bone char, granular activated carbon and ion exchange. Newer purification processes under consideration include chromatographic separation and ultrafiltration.

The "Mystery" of Direct White Sugars

In the sugar industry, beet white sugar and plantation white sugar represent two types of "direct" sugars -- that is, edible, white sugar produced directly from the expressed juice. It has been speculated that the colorants in beet sugar processing are fundamentally different from those of cane. This difference allows a very dark colored beet thick juice to be crystallized into a refined white sugar without the need for physical adsorptive removal of color, such as is required in cane sugar refining. It has been demonstrated that there is a much lower concentration of polysaccharides in beet processing and the nature of the colorants does appear to be different, of a lower average molecular weight than in the cane and less pH sensitive (Godshall, et al., 2002).

Plantation white sugar, mill white sugar, azucar directo, Blanco Directo, plantation mill white sugar, or PMWS, as it is known in the Codex Alimentarius, is an off-white cane sugar whose characteristics do not quite conform to the specifications of white, refined sugar, but which satisfies the needs of at least 50% of the world's population.

It has been reported that plantation white sugar of 80-120 IU can be crystallized from a syrup with color as high as 19,000 IU (Koteeswaran, 2002). In conventional cane processing for both raw sugar and white sugar, the color differential from syrup to crystal is in the range of 10-20:1. For beet processing, it is 100-200:1. Plantation white sugar processing can have an even higher color elimination ratio (Koteeswaran, 2002). Therefore, although there are differences between beet and cane colorants, which may account for the higher color transfer into raw cane sugar, it appears that sulfitation may change the equation for cane sugar, making the colorants behave more like beet sugar colorants in terms of color transfer. Some differences in color transfer rates are shown in Table 1.

Table 1. Color in various processes

Process	Mixed Juice	Clear Juice	Syrup	Sugar	Ratio
Beet	3405	1359	1635	23	71:1
Cane (Raw)	14,848	14,388	14,131	1005	14:1
Plantation*, Nov-June	62,287	22,608	19,313	169	139:1
Plantation*, Aug-Sept	27,555	19,578	17,753	124	143:1

* Double sulfitation, color is given for sulfited syrup

Ozone in the Sugar Industry

Ozone was discovered in 1785, and has been used in various capacities over the centuries. At the present time, there is more use of ozone in Europe than in the United States. The main use of ozone is in disinfection, particularly of water. It has also been explored to remove colored wastes from water, such as textile dye wastes. In 1992, ozone was approved for bottled water purification in the United States, and in 1997, ozone received GRAS (Generally Recognized As Safe) status from the U.S. Food & Drug Administration (FDA), which means it can be used in food processing. Again, the main use is for disinfection and microbial control on food surfaces. In recent years, because of environmental concerns, interest has intensified in the use of ozone as an alternative to chlorination for water treatment. According to a recent review on ozone (Graham, 1997), the half life of ozone in water is short, ranging from seconds to a few hours, depending on the demand of organic compounds present in the water, so its use may be considered a process rather than a food additive. The same article states that the by-products of ozone are similar to normal oxidation products found in foodstuffs.

According to Rice, et al. (2002), "Ozone may be used safely in the treatment, storage and processing of foods, including meat and poultry in accordance with the following prescribed conditions: The additive is used as an antimicrobial agent in the gaseous or aqueous phase in accordance with current industry standards of good manufacturing practice."

A benefit of ozone is that it is generated on site as needed, eliminating the need for storage. The development of larger, more efficient ozone generators has made the process less expensive in recent years. In the sugar industry, issues of proper contact are important as well as controlling the excessive foaming caused by ozone.

A combination of ozone and hydrogen peroxide, known as Peroxone, is under investigation as a powerful disinfectant for water and a possible replacement of chlorine and chloramine disinfectants. Hydrogen peroxide synergistically increases the effectiveness of ozone action.

Ozone is very reactive, and it is difficult to predict how it will react in the presence of organic matter. Questions related to the formation of unidentified oxidation products and the return of color over time, have not been fully answered yet. Total oxidation of an organic compound by ozone will lead theoretically to water and carbon dioxide as the sole products (as long as the compound does not contain nitrogen or sulfur in the molecule). Ozone may oxidize or ionize, or spontaneously decompose to oxygen and free radicals (Kim, et al., 1999). Among the most common reactions of ozone are the cleavage of carbon-carbon double bonds to produce carbonyl compounds (Figure 1) or alcohols, or addition across carbon-carbon double bonds, which results in the decolorization of dyes, such as indigo (Burke and Danheiser, 1995). Aromatic compounds are less reactive toward ozone than alkenes and alkynes. Also, different substituents on aromatic groups can have an inhibitory or enhancing effect on ozone action, depending on whether the substituent group is electron donating or electron withdrawing (Burke and Danheiser, 1995). The variable reactivity of ozone to different compounds will help to explain why it is more or less effective under different conditions in decolorizing raw sugar.

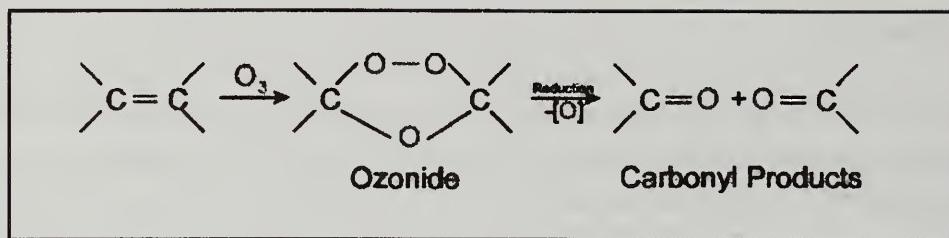


Figure 1. One of the fundamental reactions of ozone.

An interesting observation was made by Labbe, et al., (2001) when using ozone to disinfect maple sap. They found that the addition of as little as 3% sucrose to a solution markedly reduced the effectiveness of the ozone. They concluded that ozone could not be used successfully to reduce the microbial population in maple sap due to the interference by sucrose.

Although there has been interest in ozone as a decolorizing agent for the sugar industry in Cuba (Fernandez, et al., 1991; Castaneda-Perez and Dorta-Contreras, 1988), the Ukraine (Potapov, et al., 2002) and for decolorizing molasses fermentation wastewater in Spain (Peña, et al., 2003), the bulk of research on ozone in the sugar industry has taken place in South Africa. After extensive lab scale experimentation with ozone decolorization of various refinery liquors (Davis, 1996), the process was scaled up for evaluation at two carbonatation/sulfitation refineries (Davis et al., 1998). The trials at the Malelane refinery reportedly were very successful (Davis, et al., 1998; Moodely, et al., 1998), with noticeable improvements in refined sugar color, but the trials at Ubombo Sugar Refinery were disappointing, with no significant improvement in refined sugar color (SMRI, 2001).

The behavior of ozone toward colorants in cane sugar process liquors (refinery) was observed to be unpredictable. This is probably because ozone has differential reactivity toward different types of colorant molecules. According to a report (SMRI, 1996), ozone decolorized refinery liquors rapidly, with the most efficient attack appearing to be against those colorants that were most pH labile (that is, had a higher color at pH 9 than at pH 7). Raw melt that was ozonated before carbonatation produced significantly lower sugar colors than melt that had not been ozonated. The work in South Africa led to the conclusion that it was essential to follow ozonation with a precipitation/defecation process, such as carbonatation and filtration, to obtain the maximum benefit of ozonation and to prevent color from re-forming during pan boiling (Davis, 2001).

Ozone use in the sugar industry may be limited to polishing procedures or to enhance color removal during difficult times, such as when raw sugar quality is poor. Sugar Processing Research Institute has explored the use of ozone to remove off-odors from beet sugar.

Continued ozone reaction will produce aldehydes and alcohols and eventually carboxylic acids. Production of carboxylic acids in sugar decolorization causes the pH to drop slightly, but also helps to assure that color will not re-form, as the acids are less reactive during boiling than are the carbonyls.

Hydrogen Peroxide in the Sugar Industry

The use of hydrogen peroxide in the food industry in the United States is covered in the Code of Federal Regulations, Title 21, Vol 3, Chapter 1, Sect 184.1366, for specific uses, including as an

antimicrobial in milk and starch, as an oxidizing and reducing agent in dried eggs and wine, as a bleaching agent for emulsifiers, tripe, beef feet, herring, instant tea and colored cheese whey, and to remove sulfur dioxide from wine vinegar, starch slurry and corn syrup, in amounts ranging from 0.04% to 1.25% or, in some cases in the “amount sufficient for the purpose.” The same document specifies that any use of H₂O₂ not in full compliance with the established limitations requires a food additive regulation. This could limit its use in the United States. For example, a petition by Basic Vegetable Products, L.P., to FDA for the use of processing dehydrated onions with hydrogen peroxide to reduce the microbial load, was denied as being outside the scope of the regulatory framework (FDA, 1999).

Hydrogen peroxide is a powerful oxidizing agent. Intracellular hydrogen peroxide is responsible for the degradation of lignin and cellulose by the family of white rot fungi. Reactions of hydrogen peroxide are catalyzed by metal ions, especially iron, which causes decomposition of the hydrogen peroxide, producing the very active hydroxyl radical, a process known as Fenton chemistry. It is environmentally benign since it decomposes easily and completely into water and oxygen. Oxidative reactions and degradations of carbohydrates caused by hydrogen peroxide have a long history of study (Isbell and Frush, 1977, 1987; Pigman and Horton, 1972). Under highly alkaline conditions (1.2 M KOH), 600 ppm H₂O₂ at 4° C, disaccharides can be completely oxidized to formic acid within 24 hours. Fortunately, the reaction of H₂O₂ with sugar colorants is much more rapid so that under sugar processing conditions, little or no sucrose degradation occurs.

There has been longstanding interest in hydrogen peroxide as a processing aid in the sugar industry. A U.S. Patent granted in 1937 refers to interest in hydrogen peroxide to purify sugar solutions dating back to 1860, mainly for beet juices, although without much success (Reichert and Elliott, 1937). The patent was for a raw sugar refining process using hydrogen peroxide during clarification to destroy 30-90% of the coloring matter, so that the load to the bone char filters would be much less, increasing the time between regeneration. The method does not seem to have been implemented.

A literature review on more recent use of hydrogen peroxide in the sugar industry was compiled by FMC Corporation in 1975, in which 30 references, including patents, from 1970 to 1975 were abstracted, covering a wide range of products and processes (FMC, 1975). Riffer discussed the use and action of hydrogen peroxide in the sugar industry (Riffer, 1980).

Hydrogen peroxide was implemented as a processing aid to refine sugar in Venezuela in the late 1980s (Cordovez, et al., 1991), and is still in use. The refining system originally consisted of decolorization with hydrogen peroxide, melt phosphatation and filtration through Tate and Lyle deep bed filters, producing a sugar that met the standards for Venezuelan refined sugar. Changes in the Venezuelan sugar industry in 1993 necessitated higher standards for refined sugar, and the system was updated (Mendoza and Espejo, 2002). Ion exchange was introduced, but hydrogen peroxide decolorization was retained because it removed certain colorants that the resin could not. Every effort is made to make sure that all the H₂O₂ is destroyed prior to the liquor going onto the resin columns. One of the authors has stated that under these conditions, the color destruction by H₂O₂ is irreversible, there is little destruction of invert sugar and no loss of sugar due to peroxide. Furthermore, previous problems with high starch, which had required the use of amylase, were reduced due the use of H₂O₂, and, additionally, they found that H₂O₂ was particularly effective in dealing with colorants from deteriorated cane (Mendoza, 1997).

Work at Sugar Processing Research Institute showed that hydrogen peroxide, in conjunction with lime clarification and membrane ultrafiltration, would produce a sparkling clear, low color clarified cane juice (Duffaut and Godshall, 2002). However, the color transfer coefficient on crystallization remained the same as for standard cane sugar crystallization, in the range of 10:1.

In recent years, interest in hydrogen peroxide in sugar processing has been particularly intense in South Africa and India. Preliminary investigations in South Africa showed that, while H₂O₂ would not be feasible as the sole decolorizing agent because of cost, its use in conjunction with other processes, such as sulfitation, carbonatation or phosphatation, could be quite efficient and lead to lower costs (Moodley, 1992). Trials were subsequently conducted at a refinery, which showed that although the first and second sugar colors were not much changed, there was a significant drop in the colors of the third and fourth sugars, resulting in lower color of the packaged sugar (Davis, et al., 2000). The two main effects seemed to be preventing color increases from raw melt to the liquor and reducing color increases during pan boiling.

In India, part of the impetus for studying hydrogen peroxide in processing is the hope that it can be used to eliminate or reduce the use of sulfite. The effect of H₂O₂ on cane juice constituents was studied (Mane, et al., 1992). Whereas liming alone had little effect on amino acids, polyphenols and reducing sugars, following treatment with 0.1% H₂O₂, an average of 11.5% amino acids, 45.8% polyphenols and 36.6% reducing sugars were removed. Although starch was variably removed during lime clarification (from 0 to 92.6%), H₂O₂ removed, on average, 55% of the starch remaining after liming. This is consistent with the observation of Mendoza that starch problems were reduced when using hydrogen peroxide. It was concluded that sulfitation and hydrogen peroxide could be used in combination to good effect. A later study, using 10 to 50 ppm H₂O₂ confirmed the beneficial reduction of color precursors, but to a lesser degree, since considerably less H₂O₂ was used (Pachpute, 1998). Mill scale experiments in which H₂O₂ was added to A massecuite before dropping the pan significantly improved the color of the sugar, as well as its keeping qualities and resulted in lower SO₂ use (Mane, et al., 1999, 2000).

Hydrogen peroxide provides a benefit in sulfitation processes because it oxidizes sulfites to harmless sulfates. It is cheaper than ozone, less hazardous, perhaps more predictable in its behavior and does not cause the foaming problem of ozone. It also appears that colorants and color precursors are permanently destroyed and thus do not have the ability to form again on storage. Any use of hydrogen peroxide in the sugar industry in the United States, however, will need to consider if its use falls within the regulatory framework.

Sulfitation in the Sugar Industry

SO₂ plays several important roles in sugar production:

1. Inhibits non-enzymatic color formation. This occurs when a bisulfite addition product forms with aldehydes, preventing aldehyde reaction (ie, reducing sugar reaction) with amino acids in the Maillard browning reaction. In cane sugar production, since pH is generally lower than 7.0 and amino acid content is low, these types of reactions are probably not as prevalent as is often claimed.

2. Inhibits enzymatic color formation. Work on cane and beet juice indicates that enzymatic color formation produced by the browning and polymerization of phenolics by phenol oxidases and tyrosinases can account for as much as 50 percent of the color in both cane and beet juice (see, for example, Goodacre and Coombs, 1978; Gross and Coombs, 1976).
3. Besides inhibiting color formation, either of the chemical or enzymatic type, SO₂ also has a bleaching effect on existing colorants, due to little understood reduction reactions.
4. Acts as a biocide.

In earlier work by SPRI, it was shown that the effect of SO₂ is variable, depending on the quality of the cane juice (SPRI, 2001). In a model system consisting of 15 brix pure sucrose, treatment with 30 ppm SO₂ at 85°C resulted in 0.6% sucrose loss in 30 minutes, whereas HCl added to reduce the pH to an equivalent amount resulted in only 0.01% sucrose loss. No residual SO₂ could be detected only a few minutes after dosing. The same solution, after one hour, had 1.6% sucrose loss in the SO₂ solution and 0.03% sucrose loss in the HCl solution.

However, in mixed juice and clarified juice, in order to achieve a pH drop after liming and to see a color decrease, SO₂ had to be increased to 1200 ppm for clarified juice and 4700 ppm in mixed juice and the treatment time extended to 2 hours. No sucrose degradation occurred during juice treatment.

Macromolecules in the Sugar Industry

Macromolecules in the sugar industry consist mainly of various polysaccharides and some colorants; protein content tends to be low and effectively removed during clarification. Research by Sugar Processing Research Institute and others has shown that high molecular weight colorant is more likely to transfer into the crystal, as is polysaccharide. Cane juice and cane processing have much higher levels of polysaccharides than beet processing (Godshall, et al., 2002). It has also been demonstrated that the indigenous cane polysaccharide (ISP) has colorant molecules (polyphenolic acid pigments) esterified to it (Godshall, et al., 1998). One approach to improving color elimination in cane sugar processing would be to decrease the amount of polysaccharide-colorant complex in cane juice, by some means, such as by enzymatic attack, by membrane ultra-filtration, or attack by an oxygen active compound, to lower the color transfer rate. In a recent study H₂O₂ removed about 35% of color from clarified cane juice but did not have any effect on polysaccharide concentration (Duffaut and Godshall, 2002).

EXPERIMENTAL

Experimental Approach - Effect of H₂O₂, Ozone and SO₂ on Cane and Beet HMW.

Since the high molecular weight colorants (HMW) are implicated in transfer of color into sugar crystals, we decided to examine the effect of the oxygen actives on these constituents. In earlier work in this laboratory, the macromolecules were isolated by dialyzing a sugar solution to eliminate the sucrose. In this study the dialysis step was eliminated and colorants in the juice were examined directly by gel permeation chromatography (GPC). Visualizing colorants in the UV at 270 nm prevented interference by sucrose.

Ozone generation. Ozone was generated from ambient air by a small bench-top ozone generator. Ozone was pumped into the sugar solution through a sparger at 0.8 liters/min, or 3.3 standard cubic feet per hour (SCFH), to deliver about 0.075 g ozone per hour.

Sample treatment. Mixed raw cane juice, clarified cane juice, and beet thick juice were treated as described. Beet thick juice was diluted to about 15 brix (as a surrogate for thin juice, which was not available).

(a) Hydrogen Peroxide. 150g juice was adjusted to pH 8.5 with dilute NaOH, heated to 80°C. Approximately 1000 ppm H₂O₂ (30% H₂O₂ solution) was added and reacted with stirring for 2 min, then allowed to cool before GPC analysis.

(b) Ozone. 100g juice was adjusted to pH 7.0 with dilute NaOH, heated to 40°C; ozone bubbled through for 1 hr (900-1000 ppm ozone); 1-2 drops of octanol were added to control foaming.

(c) Sulfite. 150g juice adjusted to pH 8.5 with dilute NaOH, heated to 80°C; SO₂ bubbled through for approximately 10 sec (until color change noted), approximately 1000 ppm SO₂ injection.

Mixed cane juice. Mixed cane juice samples were treated with lime and a commercial acrylamide flocculent. Juice was heated to 85°C and lime and floc added together, then treatment with ozone, H₂O₂, or SO₂ was carried out as above.

Gel Permeation Chromatography. Prior to chromatography, the juice samples were diluted and filtered on a 5.0 μ filter. Colorants were separated using a Waters Breeze system, using a Waters Binary HPLC Pump Model 1525, Autosampler BioRad Model AS-100 HRLC Automatic Sampling System, Waters 2487 Dual Wavelength Absorbance detector, and Waters 2410 RI detector. Columns used in series, were Waters Ultrahyrogel Linear, Ultrahyrogel 1000, and Ultrahyrogel 500 all 7.8 X300 mm, protected by an Ultrahyrogel guard column. The eluent was 0.02M Tris buffer, pH 8.53, with peak detection at 270 nm.

Synthetic Colorants. To test the action of ozone on colorant types, three synthetic colorants were prepared according to the methods of Shore, et al. (1984). These were sucrose thermal degradation color (caramel), acid Maillard color (glycine colorant) and alkaline Maillard color (lysine colorant). Colorants were reacted for 24, 48 and 72 hours. The colorants were added (10 ml of the caramel and acid Maillard and 1 ml of the alkaline Maillard) to 500 g of a 40 brix refined sugar solution and treated for 20 min at room temperature with ozone, for a total delivery of 0.025 g ozone.

RESULTS AND DISCUSSION

Effect of Ozone on Raw Cane Sugar Color.

A raw cane sugar solution, 43.5 brix, 50 ml, was treated with ozone for 5, 10, 15, 20 and 25 min. Because of foaming, a small amount of octyl alcohol was added as an anti-foam agent, and it was noted that the octyl alcohol inhibited the effect of ozone. The results are shown in Table 2.

Table 2. Effect of ozone on color of raw sugar solution. Effect of octyl alcohol and air.

Treatment Time	Color of Raw Sugar Solution		
	Ozone only	Ozone + Octyl Alcohol	Octyl Alcohol + Air
0	3366	3366	3294
5	2813	3125	3265
10	2398	2849	3235
15	2091	2756	3236
20	1913	2700	3245
25	1837	2617	3260
Total Color Removed	45.4%	22.3%	1.3%

The data in Table 2 show that the octyl alcohol anti-foam decreased color removal by more than 50%, indicating competition for the ozone. Air sparging in the presence of octyl alcohol alone had no significant effect on the color. In repeated experiments, color removal of the raw sugar solution was consistently 40-45%

Effect of Ozone on Synthetic Colorants.

Table 3 shows the effect of ozone treatment on three types of synthetic colorants created by reacting for different periods of time. (See Experimental for details.) Although there was a moderate amount of variation in color removal, the average over-all color removal was 50%, not too different from that of the raw sugar solution discussed above. These results may help to explain, in part, why ozone has been said to be inconsistent in color removal, as the color removal may depend on the type of colorant and its ease of oxidation.

Table 3. Effect of ozone treatment on synthetic colorants.

Type of Colorant	Reaction Time	Color of Solution	% Color Removed
Glycine	24 hrs	29	36%
	48 hrs	59	80%
	72 hrs	62	53%
Caramel	24 hrs	28	75%
	48 hrs	62	51%
	72 hrs	103	42%
Lysine	24 hrs	182	42%
	48 hrs	556	35%
	72 hrs	732	28%

Preliminary Results on Juice.

Table 4 shows some preliminary results on treatment of clarified cane juice. GPC was not done on these samples.

Table 4. Effect of treatment of clarified cane juice on color and total polysaccharides.

Treatment	Color	% Removed	Polysaccharide	% Removed
Clarified juice	11,487	--	3904	--
Ozone, 500 ppm	9381	18	4055	0
H ₂ O ₂ , 1000 ppm	6327	45	3936	0
ABS, 5 ml/100 ml, RT	6174	46	3434	12
ABS, 5, ml/100 ml, 80°C	7225	37	3421	12
UF 300 kDa	10,555	8	3597	8

ABS = ammonium bisulfite

RT = room temperature

polysaccharide, ppm on solids

Hydrogen peroxide and ammonium bisulfite (ABS) removed similar amounts of color, but ozone removed much less. Neither ozone nor H₂O₂ had any effect on the polysaccharide level, but ABS showed a 12% decrease. ABS had a similar effect on removing polysaccharide from A molasses. Subsequent experiments with SO₂ showed that it also aided in removal of polysaccharide. The effect of sulfite on polysaccharides may be one mechanism that influences color transfer. The ultrafiltration (UF) results indicate that most colorants and polysaccharides were below 300,000 Daltons.

Effect on Juice Constituents.

Table 5 shows the effect of treatments on raw cane juice, clarified cane juice and beet thick juice. Lime and flocculent addition caused a large removal of color, turbidity and polysaccharide in the mixed cane juice -- therefore, any additional removal with a treatment agent represents the benefit conferred by that agent. With reference to mixed cane juice #1, SO₂ conferred 7.3% advantage in color removal, 1.3% advantage in turbidity removal and 10.3% advantage in polysaccharide removal. There was little effect on cane juice #2. These results would indicate that SO₂ has the potential for a significant role in the removal of macromolecules. The effect of SO₂ on polysaccharides is not seen when clarified cane or beet juice was treated, indicating that the action may be a synergistic one between lime and sulfite. It is interesting to note that all the additives had a significant effect on the turbidity of already clarified juices, indicating additional clarifying action.

Table 5. Effect of treatment, % Removed from juice

Sample	Treatment	Color	Turbidity	Polysaccharide
Mixed (raw) cane juice, sample #1	Lime + Floc*	36.9	97.1	32.6
	Lime + Fl + SO ₂	44.2	98.4	42.9
Mixed (raw) cane juice, sample #2	Lime + Floc*	54.7	98.8	39.5
	Lime + Fl + SO ₂	54.3	98.7	41.1
	Lime + Fl + H ₂ O ₂	66.8	97.6	33.4
	Lime + Fl + Ozone	27.8	88.0	27.4
Beet thick juice, diluted	SO ₂	4.8	50.0	0
	ABS	21.9	49.1	7.8
	H ₂ O ₂	57.6	44.3	0
	Ozone	48.4	59.0	2.9
Clarified cane juice	SO ₂	11.3	28.6	0
	ABS	16.8	52.9	1.0
	H ₂ O ₂	44.6	46.6	0
	Ozone	36.7	28.6	0

* Commercial flocculent

GPC Results

Figure 2 compares the GPC colorant profiles of beet thick juice (2a) and clarified cane juice (2b). Both samples represent clarified product, although the beet juice had undergone evaporation, and probably some color increase due to boiling, which the cane juice had not undergone. The samples are comparable because they have been diluted to a similar solids content. The beet sample has only about one-fourth the amount of colorant of cane (maximum absorbance of 0.30 for beet vs 1.20 for cane). Beet also has a lower over-all molecular weight profile than cane.

Figure 3 shows the effect of lime clarification on raw cane juice. The profile of the two highest MW peaks in the raw, mixed cane juice (retention times 89-92 min and 91-93 min) was changed upon liming, with a reversal of the heights. Factory clarified juice showed the same profile as that of lab clarified juice.

Figure 4 shows the effect of H₂O₂ on cane juice. In both raw cane juice (4a) and clarified cane juice (4b), the large colorant peak at 94 min was significantly reduced -- by more than two-thirds in raw juice and by about half in clarified juice. This peak has been shown in previous GPC work at SPRI to contain a large proportion of cane colorant. H₂O₂ showed no effect on the colorants in beet juice.

Figure 5 shows the effect of SO₂. The effects are similar for raw cane juice (5a) and clarified cane juice (5b) in that there appears to be a noticeable shift to a lower molecular weight of the major colorants. The beet juice (5c) was also noticeably affected by SO₂ treatment. In spite of the fact that this juice had already undergone sulfitation/carbonatation clarification and may not be expected to show many changes with SO₂, there was significant diminution of the peak at 98.8 min and some shifting to lower molecular weights.

CONCLUSIONS

Hydrogen peroxide and ozone showed little effect on the HMW colorants of beet thick juice. Further study is need to assess their effect on raw beet juice, which was not available for this study. Hydrogen peroxide considerably diminished a major colorant in cane juice. Ozone had little effect on raw or clarified cane juice. SO₂ had the most complicated effect, appearing to diminish some peaks and to cause a shift to a lower molecular weight ranges in both cane and beet. It is possible that this activity makes sulfitation an effective agent in decreasing the transfer rate of colorant into the sugar crystal.

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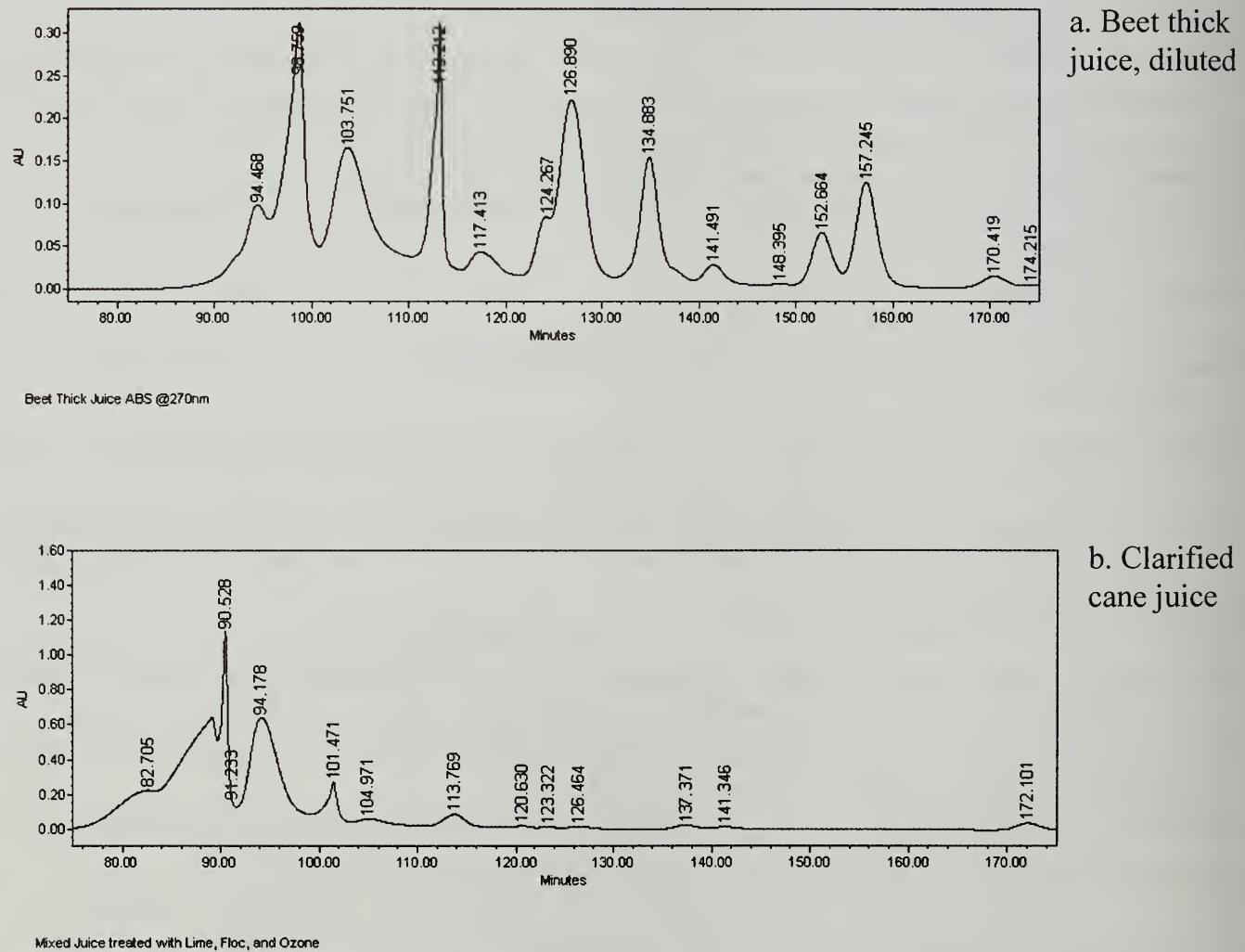


Figure 2. Comparison of the GPC profiles of beet thick juice and cane clarified juice.

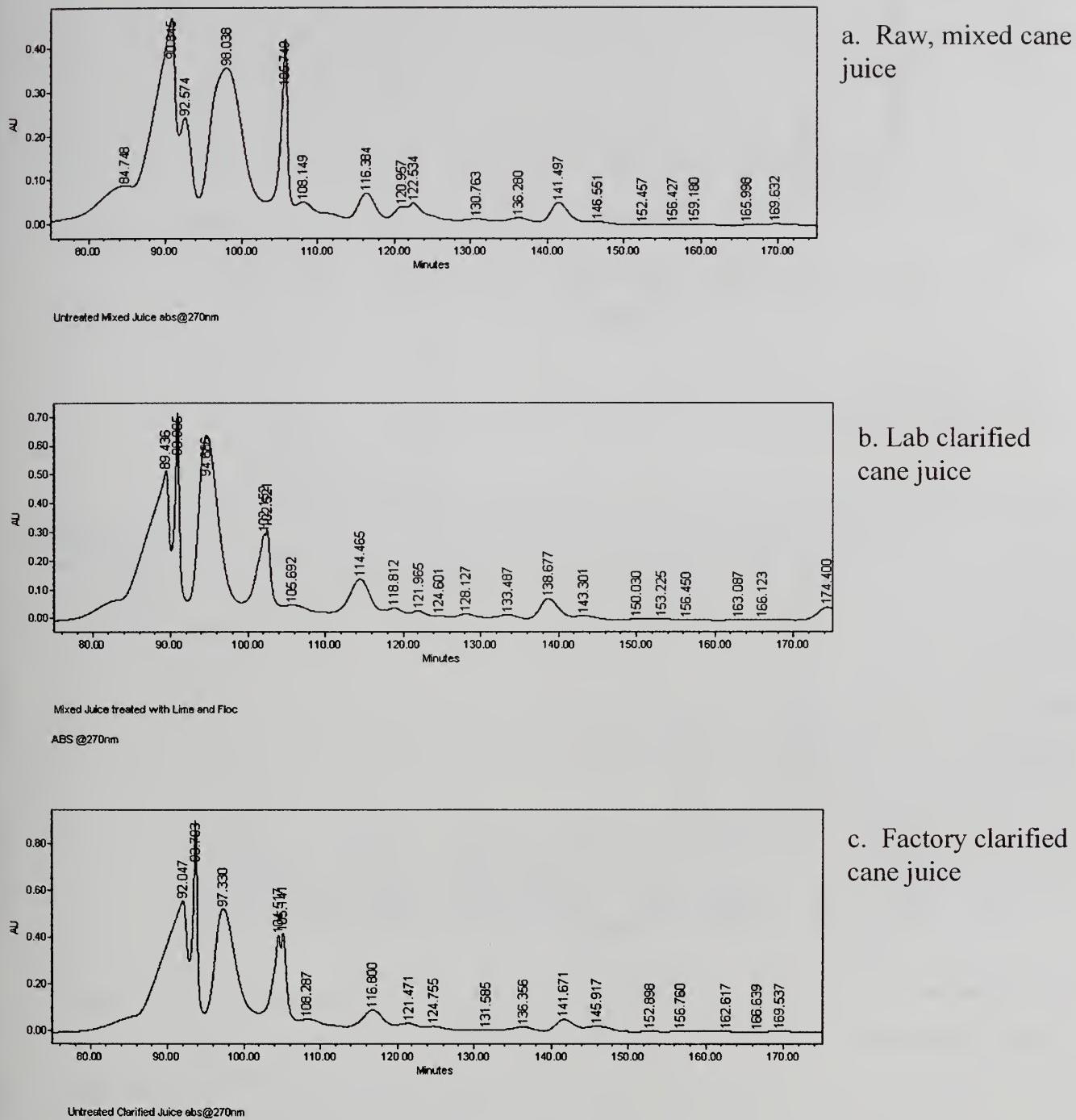


Figure 3. Changes in GPC profile after clarification. Comparing lab clarification to factory clarification of cane juice.

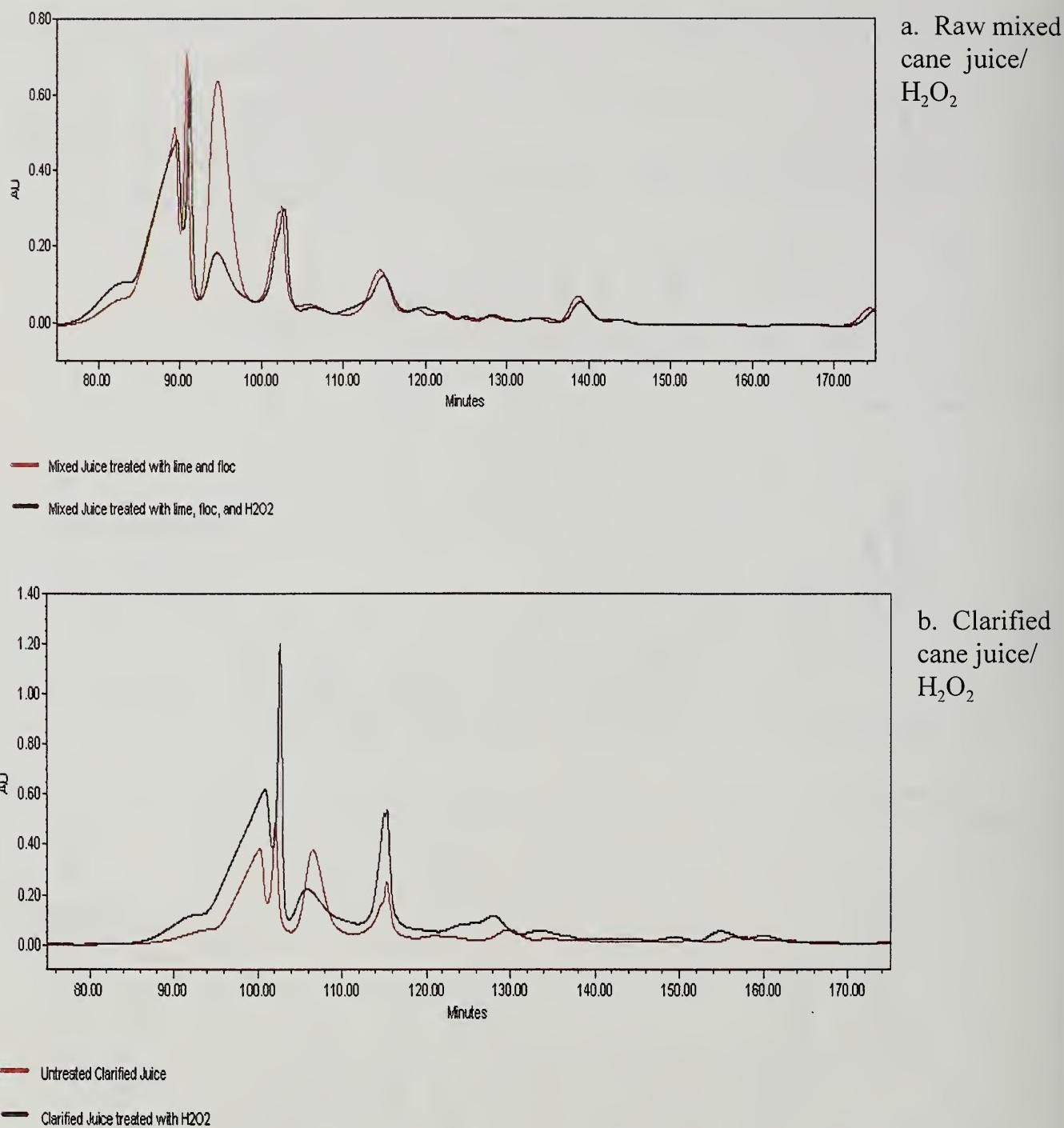


Figure 4. Effect of H_2O_2 on the GPC profiles of colorant in cane juice.

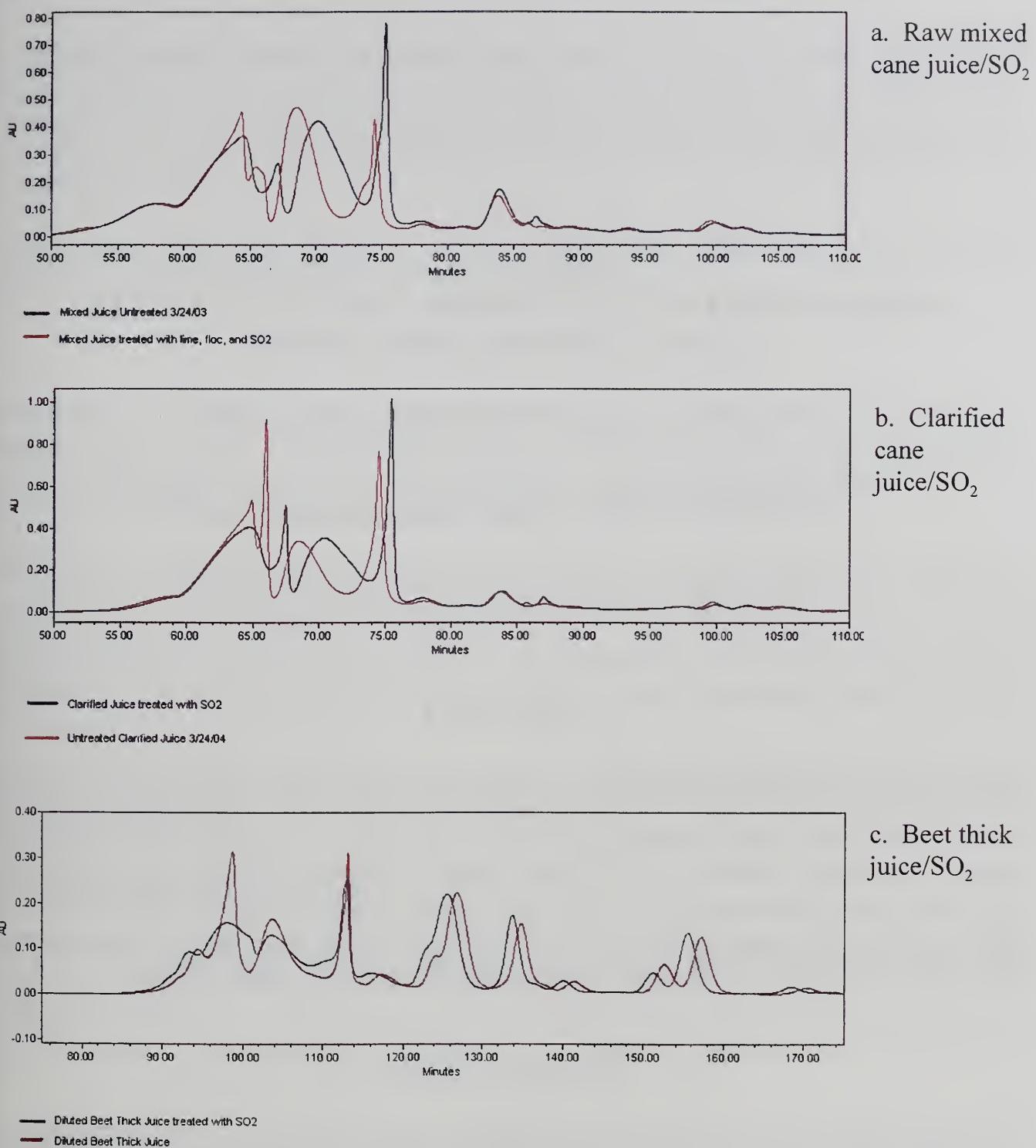


Figure 5. Effect of SO₂ on GPC profiles of colorants in cane and beet juice.

OBSERVATIONS ON SUCROSE WATER ACTIVITY - MOISTURE RELATIONSHIPS

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ABSTRACT

The sugar-water relationship on the surface of refined sucrose crystals is a complex and only partially understood phenomenon. The mechanism appears to be related to the film of amorphous sugar formed on the crystal surface during drying. This film slowly gives up moisture on standing, a condition sometimes referred to as curing. Using Equilibrium Relative Humidity (ERH) measurements the authors present a model for the curing step and it's relationship to frequently observed caking and lumping problems. They also explore the behavior of sugar in storage and some of the apparent anomalies in the measurement of moisture in freshly produced sugar.

INTRODUCTION

This should not be viewed as a report of laboratory studies performed in a research environment. Rather, it is an essay on water activity or equilibrium relative humidity and dew point as tools to evaluate the sugar crystal-surface moisture relationship. The information is a distillation of numerous observations and measurements made in the factory environment.

Measuring the residual moisture in granulated sugar has always been a difficult task, particularly in the factory laboratory environment. Moisture levels are at or beyond the limits of classical moisture analysis techniques. The sugar crystal's apparent affinity for water further complicates the picture. The objective of this work was to explore the water activity of sucrose and it's possible application as an analytical tool.

In the course of this paper we will use the term granulated sugar to refer to commercially produced, refined white sugar that has been dried to storage stable levels.

BACKGROUND

Sugar caking is a universal problem. The sugar industry has always had to contend with caking and lump formation. Sucrose has a high affinity for water and a strong propensity to form supersaturated solutions. While this latter property is a real asset when we are manipulating the crystallization process, it is a definite liability when handling and storing the finished product.

Caking results when stored sugar is exposed to high and low humidity cycles. It may happen with any sugar, but seems worse with fine granulations. Other researchers have found that caking problems become more severe as the amount of fines (-100) present increase. Our practical experience leads us to agree. Packages of the very fine granulations - Gel Gran and Bakers Special - tend to have a much shorter storage life than packages of coarser granulations stored in the same environment.

Generally recommended sugar storage conditions are temperatures below 35° C and relative humidity less than 70%.

Methods of moisture measurement

The Karl Fisher (KF) method probably provides the best measure of water present in sugar. There are problems with the method, however. KF suffers from atmospheric moisture interference. Sugar has limited solubility in KF compatible solvents. Finally, the moisture levels encountered in granulated sugar are close to the practical limits of the methodology. Despite these shortcomings KF has long been considered the reference method for moisture in sugar.

The standard gravimetric method for sugar moisture is the convection oven procedure, 4 hrs @105° C. An alternate, less frequently used method calls for 8 hrs @ 60° C in a vacuum oven. Both methods take time to run and require precise analytical technique. The range of moisture values in granulated sugar is typically 0.01 to 0.05 %. This equates to 10 to 50 milligrams in sample sizes usually encountered. Accurate measurements of such weights is very difficult under conditions typically found in a sugar factory laboratory

Unless conducted with great care, gravimetric methods are subject to high variability. They are extremely operator and equipment dependent. This variability has made sugar drying research a difficult and frustrating task. Additionally, the length of time required to obtain results limits the usefulness of classical gravimetric methods as process control tools.

In the past few decades, a series of automatic moisture balances have been developed which have the sensitivity to give reproducible results with granulated sugar. The approach most commonly used involves measuring the rate of weight loss over time and extrapolating to an endpoint. These instruments provide results in a short time and are valuable process control tools. They also give better reproducibility than traditional gravimetric methods.

The major shortcoming of all gravimetric methods is that none really measure the total amount of water in freshly dried sugar. Not all of the moisture on the crystal surface is free to be driven off during the first 24 - 48 hours. In fact, some of the surface moisture is tightly bound to the sugar crystals indefinitely.

An alternate approach to sugar moisture measurement is to examine the equilibrium moisture level - Water Activity (Aw) or Equilibrium Relative humidity (ERH) - in the air surrounding sugar crystals. This technique has been studied at the research level off and on for many years, but has seen only limited application at the plant level. Equipment ruggedness, reliability and cost have limited the application. The advent of small, recording hygrothermographs open new possibilities for the method. These instruments, when placed in the head space of a closed container of sugar provide meaningful data about the moisture levels and caking potential of the sugar.

The Aw or ERH of a substance is a function of the amount of moisture in the surrounding air at equilibrium. The Aw of a substance is defined as the ratio of the partial pressure of water vapor in the air around the substance to the partial pressure of water vapor in saturated air at the temperature of measurement. ERH is defined as $100 * Aw$.

An advantage of Aw when working with granulated sugar is the wide range of measured values compared to the observed moisture levels. Sugar Aw values typically range from 0.40 to 0.70 with a precision of ± 0.02 . Corresponding moisture measurements range from 0.025 to 0.05 with a precision of ~ 0.01 .

Aw is probably a better descriptive term, but ERH is a little easier concept to understand. Everybody is familiar with relative humidity as a meteorological concept. Both Aw and ERH are temperature dependent measurements.

Another property of air related to the ERH is the Dew Point (DP). The DP is the temperature at which water vapor present in the air sample condenses out. DP is independent of temperature as long as the ambient temperature is above the dew point. Our references to sugar's DP are more correctly the DP of the air surrounding the sugar crystals.

Figure 1 illustrates the relationships between the ERH, DP and temperature in the head space over a freshly granulated sugar sample. ERH was used instead of Aw in this example for convenience, as the scales coincide.

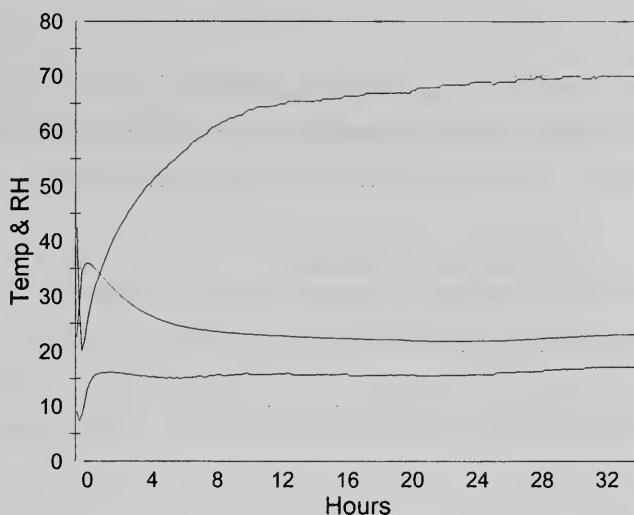


Figure 1. ERH (top), Temperature (center), and DP (bottom) curves for freshly granulated sugar.

Our experimental data indicates DP may be superior to Aw as a gauge of sugar drying. Response time is faster because of the temperature independence. The system in Figure 1 required at least 24 hours to approach ERH equilibrium, partially because of the time required for the warm sugar sample to cool to room temperature. On the other hand, the DP reached an essentially constant value in less than two hours.

Discussion of the sugar drying process

Granulated sugar leaves the centrifuge with approximately 1% moisture remaining. Very little, if any of this moisture is trapped within the sugar crystals. The interior of sugar crystals may be considered water free. It is generally accepted that the moisture present is concentrated on the surface of the crystals.

In the factory's granulator sugar is dried from 1% moisture to an apparent moisture level less than 0.05%. Most of the moisture is removed in a relatively short time. Typical granulator retention times are 30 minutes or less.

Studies of sugar drying curves show two distinct moisture release rates. The first 80 to 90% of the moisture present in or on wet sugar is removed very rapidly, presumably via an evaporation mechanism. The general shape and slope of sugar drying curves in this region correspond closely to those observed when drying insoluble materials, such as sand moistened to comparable levels.

In the vicinity of 0.1% moisture the drying rate of sugar slows markedly. Unlike the drying curves of insoluble materials, which continue to zero at essentially constant rates, sugar drying curves flatten out to a second and much slower rate. Presumably the rate-limiting step changes from evaporation to some other property, probably the forces binding water to sucrose. Many researchers have studied this phenomenon but unanimous agreement on the exact nature of the forces still eludes us. Sucrose solubility, supersaturation, and crystallization rates are probably involved, as are hydrogen bonding interactions between sucrose and water.

Sugar's Aw Anomaly

One of the first things that becomes apparent when studying the ERH or Aw properties of sugar is that the values obtained are too high for a substance with a moisture level of 0.05%. The common range of values observed, 0.5 to 0.6 is what would be expected with products in the 10 to 20% moisture range (see Table 1).

We know from KF measurements of total moisture in sugar that the observed ~ 0.05% water value is pretty good. Therefore it is reasonable to assume that the portion of the sugar crystal interacting with the surrounding atmosphere is somewhere in the 15% moisture range. If this is the case we are dealing with the outer 0.3-0.4% of the crystal's mass, possibly more in fresh sugar and less in well-aged sugar.

Table 1. Range of published Aw values for a number of substances.

Compound	Moisture Content	Aw
Saturated Sugar Solution	33%	0.83 -0. 84
Honey	15-18%	0.50 - 0.60
Raisins	15-18%	0.51 - 0.56
Pasta	12%	0.45 - 0.50
Hard Candy	2%	0.20 - 0.35
Granulated Sugar	<0.05%	0.45 - 0.55

Equipment Used in Study

The principle measuring instruments used were digital temperature - relative humidity recorders (Onset model H-8 HOBO-Pro Temp/RH Datalogger). These small, rugged and inexpensive units are designed for environmental measurements. They are approximately 75mm diameter x 50 mm high devices resembling a hockey puck. They are launched and downloaded via a computer. HOBOs do have some limitations. They are designed for non-condensing environments. They have limited RH accuracy ($\pm 3\%$), and their dew point calculations are based on a constant 1000 millibar pressure.

The sample chambers had a volume of approximately 4 liters. They were usually filled to 65 -75% of volume (typically 3 to 5 kg of sugar.). Different containers were used. The principle requirements were an opening large enough to pass the data recorder and an airtight, sealable lid.

Some laboratory Aw measurements used to check the calibration of the HOBO instruments were made with a Novasina MS1aw Water Activity meter.

Fresh sugar used in the study was unscreened production sugar dried in a Hersey granulator with an inlet air temperature normally $<90^{\circ}\text{ C}$. Conditioned and silo sugar samples were also taken from factory streams. Typical MA was 380 to 400 μ and CV was 32 to 35. Sugar ash levels were 0.010 to 0.012 and color 25 to 35. Invert was essentially zero. Typical level of fine sugar crystals (-100 mesh) was $<3\%$.

OBSERVATIONS

Wet Sugar

Refined sugar is discharged from the centrifuges at approximately 80° C and 1% moisture. This moisture is associated with a film of sugar syrup on the crystal surface. A common statement seen in the literature is that this film is saturated or supersaturated. This may not be the case. Our measurements indicate the syrup on wet sugar crystals is not quite saturated, even at room temperature. The observed Aw of a saturated sucrose syrup (approximately 67 Brix @ room temperature) is in the 0.83 - 0.84 range. Our measurements of the room temperature Aw of wet sugar yielded values between 0.92 and 0.86, corresponding to syrup concentrations of 55 to 60 Brix. Dew point values were $>25^{\circ}\text{ C}$.

Freshly dried sugar

An important point to remember is freshly dried sugar's apparent moisture as measured by weight loss on drying does not equal true or total moisture in the sugar.

The apparent moisture level in refined sugar fresh from the granulator is very low. A typical factory control target is 0.035%. Observed values range from 0.015 to 0.04%. Given the limitations of gravimetric fresh sugar moisture determinations, these reports are suspect. We have seen situations where the observed gravimetric moisture level in fresh sugar will double upon standing 12 to 24 hours in a sealed container. Also, fresh sugar will form a solid mass in a sealed container despite the fact that its observed gravimetric moisture level is essentially identical to aged or conditioned sugar that does not cake.

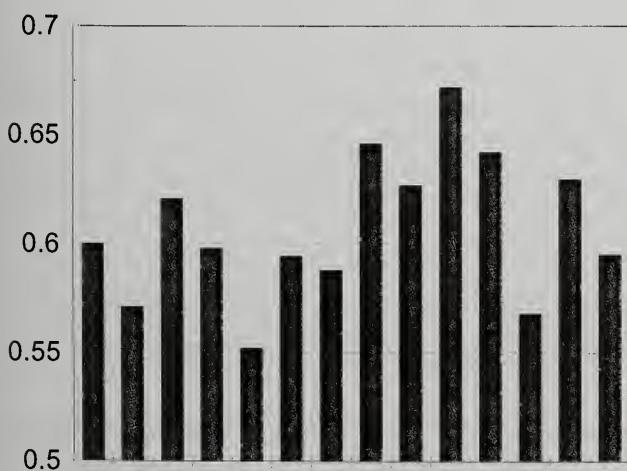


Figure 2 shows typical day to day variations in observed Aw of freshly granulated sugar. The values are fairly high and show only limited correlation with observed moisture values. This latter point is probably due to the shortcomings of the moisture measurement methodology. The Aw values observed were 0.55 to 0.70. Corresponding DP values were in the 15 to 16° range.

Fig. 2. Aw values for daily grab samples of fresh sugar.
Shows variation in production sugar Aw values.

Aw and DP values in fresh sugar seem to be related to the granulator inlet air temperature. Further re-enforcing that observation is the fact that the Aw of sugar dried at room temperature on the laboratory bench is quite a bit higher than of sugar from the heated granulator at similar observed gravimetric moisture levels. Values in the 0.82 to 0.84 range were obtained, approximating saturated sugar solutions. While this might indicate that the Aw of freshly dried sugar is a direct function of the temperature of the drying, one might also infer that the surface of a freshly produced sugar crystal has different properties than that of an aged crystal.

Conditioned sugar

To counter the caking observed with freshly produced sugar, the industry has developed the practice of aging or conditioning sugar. The goal of conditioning is to let the crystal surface film come to equilibrium with the storage environment. Two general approaches are used - 7 to 10 days retention in static bulk storage or 24 to 48 hours of purging with dry air. The beet sugar industry has primarily used the former technique and the cane industry the latter.

In a purged system the moisture released is swept from the silo. In static conditioning the moisture migrates to colder parts of silo, typically the bottom. Any lumps formed during conditioning are broken up in handling or removed when the sugar is moved and scalped.

Conditioned sugar will have observed gravimetric moisture values in the 0.02 to 0.04% range, essentially identical to fresh sugar. However it will have a lower water activity, about 0.45 to 0.55 and a dew point in the 12 to 13° range.

One of Amalgamated's factories uses a dry air purge system to condition. The conditioning silo has a two day retention and lowers the Aw of sugar into the 0.45 to 0.50 range. The air used is conditioned to an Aw of 0.12 at 24° C. Air exiting the silo has an Aw of 0.61 at 36° C. Rough mass flow calculations across conditioning silo indicate about 41 pounds of water is removed per hour. Interestingly enough, this corresponds to about 0.05% on sugar throughput.

Silo sugar

If the AW of the storage environment is lower than the Aw of the sugar, it will continue to give up moisture on standing. The driving force here appears to be temperature. A typical bulk silo is a last in first out storage device. The top portions are nearly always warmer than the bottom. The resulting temperature gradient causes moisture to migrate to the colder lower portion of the silo. This explains both the apparent additional drying observed in sugar drawn from silos and the layer of hard sugar found in the bottom of most silos.

Little or no reduction in gravimetric moisture can be measured, but silo sugar typically comes out of storage with Aw levels in the 0.35 to 0.45 range and dew points in the 7 to 9° range.

Amalgamated has installed air circulation systems in some of their silos. This circulation sweeps the moisture released by the cooling sugar out of the silo and prevents moisture buildup in the

bottoms. Sugar stored in these silos comes out free flowing and there is no buildup of hard sugar in the silo bottom. In fact, the sugar is so free flowing that the silo discharge gates must be tightened to control leakage.

Old Sugar

If bagged sugar is held in an environment where the surrounding air can circulate, the Aw will approach the Aw of the surrounding air. A search of Amalgamated's warehouses turned up a few pallets of sugar which had been stored in an unheated Inter-mountain terminal warehouse for over five years. While this is not a good warehousing practice, it did provide samples of relatively old, well-conditioned sugar. For one year during the storage period temperature and humidity monitoring recorders had been placed in this warehouse as part of a storage environment study. The average annual temperature inside the warehouse was 15°, RH 41% and dew point 1°. The old sugar stored under these conditions was free flowing and indistinguishable from sugar less than one year old. It had an Aw of 0.21 (21% ERH) and a dew point <1°. Here at least, the dew point has a greater impact on sugar Aw than the RH. One might assume that the shelf life of sugar is infinite if it is stored in this kind of environment.

The following table summarizes our observations.

Table 2. Summary of typical dew point and Aw values for various sugars

Sugar Evaluated	Dew Point	Aw 25°C
Wet Sugar	>25	0.86-0.92
New Sugar	16	0.59
Conditioned	12.5	0.45
Silo Sugar	8	0.34
Old Sugar	1	0.21

Sugar in Transit

Bulk sugar being transported in a rail car sees thermal cycling. In particular, the head space temperature swings widely (the head space is typically about 25% of total car volume). Head space temperature swings result in corresponding pressure changes inside the car. Typical bulk rail cars breath to some extent as a result. Most Gravity Pneumatic (GP) cars have a vented head space. Airslide (AS) cars have sealed hatches but most now in service have leaking bottom flanges. Warmed air leaves the car each day, to be replaced by outside air as the car cools the next night. Depending on ambient conditions, this can result in a removal of moisture from the sugar or a moisture gain.

The Pressure Differential (PD) cars used by Amalgamated are unique in that they are essentially airtight and do not breath like GP and AS cars. The sealed nature of the PD car provides an opportunity to study the behavior of sugar Aw in a sealed environment subjected to thermal cycling.

Figure 3 shows the diurnal variation of headspace conditions inside a sealed PD type car of sugar. In this case the DP dips each night as the ambient temperature drops below the DP. Presumably moisture is condensing on the walls of the car at this point.

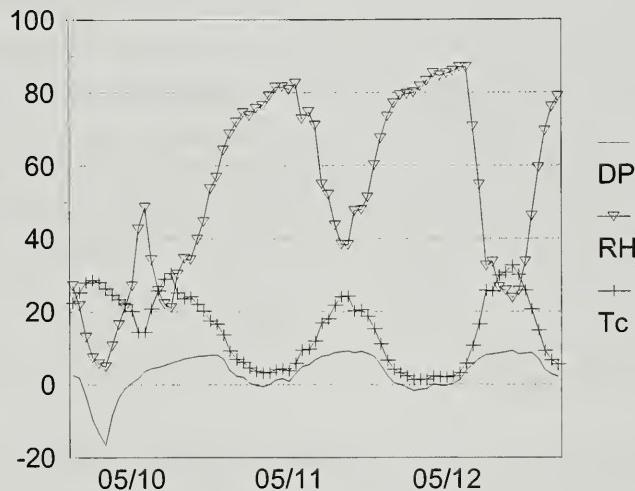


Fig. 3. Dew Point (DP), Relative Humidity (RH), and Temperature (Tc) in a loaded, sealed RR car.

The initial dew point dip to -18° shown is due to post loading purge with dehumidified air. Dew point stabilizes at 5° within 24 hours after loading, but ERH takes two days or more to come to a stable maximum. The temperature and humidity values stabilized and remained similar to the third day's values for the balance of the car's trip.

While the ERH fluctuates wildly during diurnal temperature cycles the DP stays relatively constant, only dipping as the dry bulb temperature drops below the daytime DP. Presumably condensation is taking place on the car walls.

Additional thoughts on the nature of the crystal surface film.

One aspect of sugar moisture studies that is sometimes overlooked has to do with the nature of freshly granulated sugar. It is very difficult, if not impossible, to duplicate the surface conditions found on freshly produced sugar crystals in a laboratory environment. For this reason, moisture studies almost have to be conducted adjacent to a producing factory where samples can be taken directly from the process stream and evaluated immediately.

A number of theories have been postulated as to the fate of the sugar in solution on the surface of wet sugar crystals and the exact nature of the surface film. It has been variously described as a super-cooled liquid layer, a glaze overlying a supersaturated syrup film; as a hard, glassy layer, or a porous

amorphous layer. None of these models appear to exactly fit the observed behavior of granulated sugar. Perhaps what we are seeing is just water molecules bound to the surface of the solid crystal.

Early in our work we theorized that the surface film was an amorphous glass, similar to hard candy. Experimental data showed this was not the case, at least it isn't a simple sucrose glass. A batch of hard candy boiled to 160° C was made in the laboratory and the Aw measured. The values observed, 0.20, were much lower than those seen with granulated sugar. This observation was confirmed in conversations with a candy technologist. Hard candies are reported to typically have moisture contents of about 2% and Aw in the 0.20 to 0.30 range.

If water is added to aged or conditioned room temperature granulated sugar, even in very small amounts, the Aw will go to 0.83, the value of a saturated sugar solution. The above would lead one to infer that the Aw of granulated sugar is a function of the driving force applied during drying or possibly the nature of the surface of freshly granulated sugar.

One possibility is the use of the Aw technique to study the exact nature and concentration of the syrup film on sugar from centrifuges. Preliminary results are very intriguing. They indicate the syrup film is not saturated (65 to 70 Bx) but probably closer to 50 Bx.

We anticipate doing further work to incorporate Aw or DP as sugar production QC tool.

IN SUMMARY

Freshly dried sugar is not yet dry. Conditioned sugar still contains measurable amounts of moisture.

Aw, ERH and Dew Point are useful moisture monitoring tools. DP appears to be especially useful.

Sugar should be <0.4 Aw and <10° DP prior to packaging or shipment to prevent caking and lump formation.

DEXTRAN IN REFINED SUGAR: IMPACT ON HARD CANDY PROCESSING

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ABSTRACT

Dextran content in sugar is a major concern for end users such as candy manufacturers. Contamination of the sugar with dextran, above a certain ppm level, will affect hard candy processing. The impact is measured in changes in candy thickness/weight and is related to dextran content in sugar. We report on the monitoring of dextran in refined sugar, over the course of several months, using the Midland Sucrotest monoclonal antibody method. We correlate dextran content in refined sugar with changes in hard candy weight and geometry. Data suggest that dextran content in refined sugar is related to the amount in raw sugar used in refining. Estimates of dextran removal during the refining process are given.

INTRODUCTION

Refined sugar is the key ingredient for the manufacture of hard candy. It plays a strong functional role in the flavor, texture and appearance of hard candy. The functional performance of sugar is affected by its purity, especially with regard to high molecular weight polysaccharide contaminants like dextran. Dextran, Figure 1, is a high molecular weight, branched glucose polymer synthesized by the *Leuconostoc* soil bacterium found in and around sugar cane and juice. There have been reports of hard boiled candy distortion on high speed die cutting machines where the candy rope "pulls away" from the die cut as the candy cools, leading to packaging problems and high reject rates (1-3). Very close tolerances in candy thickness and weight, in order to "fit" the packing equipment, require control of product size and shape. A thickness variation of 2 millimeters causes problems in certain types of packaging. We report on the study of the effect of dextran on candy weight and geometry, using an analysis method to measure and

monitor dextran in refined sugar. We correlate dextran content in refined sugar with changes in hard candy weight and geometry. Estimates of dextran removal during the refining process are given.

METHODS

Three methods of measuring dextran in sugar are listed in Table 1 along with our assessment of ease of testing and expense. All three methods are used to measure the higher amount of dextran found in raw sugar, but none are used to measure dextran in refined sugar. We chose to use the newer Midland Sucrotest antibody method for measurement of dextran in refined sugar for this study because it was simpler to execute and was specific for dextran.

Procedure:

A few modifications were made to the test procedure in order to adapt the dextran Sucrotest to give greater sensitivity for measurement of lower concentrations of dextran in refined sugar. We dissolved the refined sugar to give a 40 brix solution. The liquid sugar sampling volume was increased from 10 microliters up to 50 microliters. The antibody was dissolved into a 12 ml volume of buffer instead of 6 ml. The materials needed for testing and the modified method are specified below. A diagram of the Midland Sucrotest procedure can be found in Figure 2.

Materials:

Three dextran standards: 68K, 200K, and 2M MW (Sigma, St. Louis, MO) where used to establish the standard curve of turbidity as a function of dextran concentration and molecular weight. We choose the two million molecular weight standard for use in these studies. A monoclonal antibody test kit was purchased from Midland Research Laboratories, Inc., Lenexa, KS and contained Monoclonal Antibody (M9010), freeze dried; phosphate buffer powder (M9008), pH 7; membrane cartridge filter (M9000), 0.45 micrometer pore size; cuvettes and a turbidimeter (nephelometer) (M9020).

Sample preparation:

For the calibration curve a two million molecular weight dextran standard was dissolved in distilled water to make solutions with concentrations in the range of 10 to 150 ppm. For test samples, sugar or hard candy was dissolved in distilled water (40% w/w), stirred magnetically for an hour, and then filtered with 0.45 micrometer membrane cartridge filter before measurement.

Antibody preparation:

Prepare buffer solution (pH 7) by dissolving one vial of phosphate buffer powder in 100 ml distilled water. Add 6 ml of buffer solution in antibody vial, shake gently, then let it sit until the antibody is dissolved. Pour the solution out. Add another 6 ml of buffer solution to rinse the vial.

Combine the two 6 ml solutions (12 ml of buffer/vial in total) and mix them well and let stand for an hour before the test.

RESULTS

The amount of dextran was measured in refined sugar using the Midland Sucrotest method for dextran. Results of the testing for two sugar refiners are showing in Table 2. Typical values for dextran in refined sugar ranged from <10 ppm up to > 200 ppm. Some differences in the average levels of dextran were noted between the two sugar refiners.

An experimental design trial was conducted at the plant to determine the effect of dextran on hard candy manufacturing. Control candy was made with 20-ppm dextran (dry sugar weight basis) for six different candy flavors. The effect of dextran on candy thickness, diameter and weight was measured. Higher levels of dextran were tested by adding dextran (Sigma, Industrial grade, average molecular weight 5 million to 40 million) to the sugar, at levels of 250 ppm (dry sugar basis) or 500 ppm (dry sugar basis), as it is liquefied and cooked during processing. The test variables and number of runs are listed in Table 3.

Dextran affects the amount of broken and rejected product. The extent of dextran impact on candy line breakage and removal due to deformity is mediated by the type of flavor, as shown in Table 4. For flavor A, the amount of line breakage and misshapen candy removed from the process nearly doubled from 2.3% up to 4.2% when dextran increased from 20 ppm to 500 ppm. For flavor C, the amount of line breakage and misshapen candy tripled from 1.2% up to 3.7%.

Dextran affects candy thickness and diameter. Very close tolerances in candy thickness and weight are required in order for the candy to "fit" the packing equipment. A candy thickness variation of 2 millimeters causes problems in packaging. Listed in Table 5 are the measured candy thickness (height), weight and diameter. At a target candy weight of 32 g, with 500 ppm dextran, the thickness of the candy is found to exceed control candy thickness by at least 2 mm (Flavor A) or 2.4 mm (Flavor C). When candy thickness or height of candy pieces is expressed as a ratio of the height divided by the weight, a linear relationship is observed between the height to weight ratio of the candy and the level of dextran as shown in Figure 3.

Average package weight is plotted as a function of the amount of dextran. The weight of the candy package drops below the target weight of 32 g at dextran levels above 250 ppm (dry sugar basis) as shown in Figure 4. Packages that are underweight cannot be sold.

Dextran Removed During Refining

Refiner A, running a typical refining process, using phosphatation in the process, measured dextran in raw sugar received into the refinery over the course of about six months. During this same period, dextran is measured in the refined sugar received into the candy processing plant from refiner A. The data in Figure 5 show that significant purification of the sucrose crystals occurs during refining. It appears that dextran removal during purification is dependent upon dextran level in raw sugar, with better removal of dextran at lower dextran levels in raw sugar coming into the refinery. We estimate about 50% of the contaminating dextran is removed if the content of dextran in raw sugar does not exceed 250 ppm. Working with sugar suppliers, dextran

can be controlled to 125 ppm or less for sugar sent to candy manufacturing. Figure 6 shows that, although the dextran content in the refined sugar fluctuates, levels of dextran are controlled to 125 ppm or less.

DISSCUSSION

Dextran levels in sugar above 125 ppm lead to significant changes in candy geometry and appearance. Candy height/thickness increases along with a decrease in diameter and a rough/irregular surface appearance. Geometry changes prevent candies from meeting package size and weight requirements resulting in product rejection and high cost to candy manufacturing. Sugar refining removes significant amounts of dextran, providing the raw sugar is not heavily contaminated (i.e. < 250 ppm). Dextran level in refined sugar fluctuates but can be controlled by careful monitoring of the raw sugar and the refining process.

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Figure 1. Diagram of dextran molecule.

Dextran

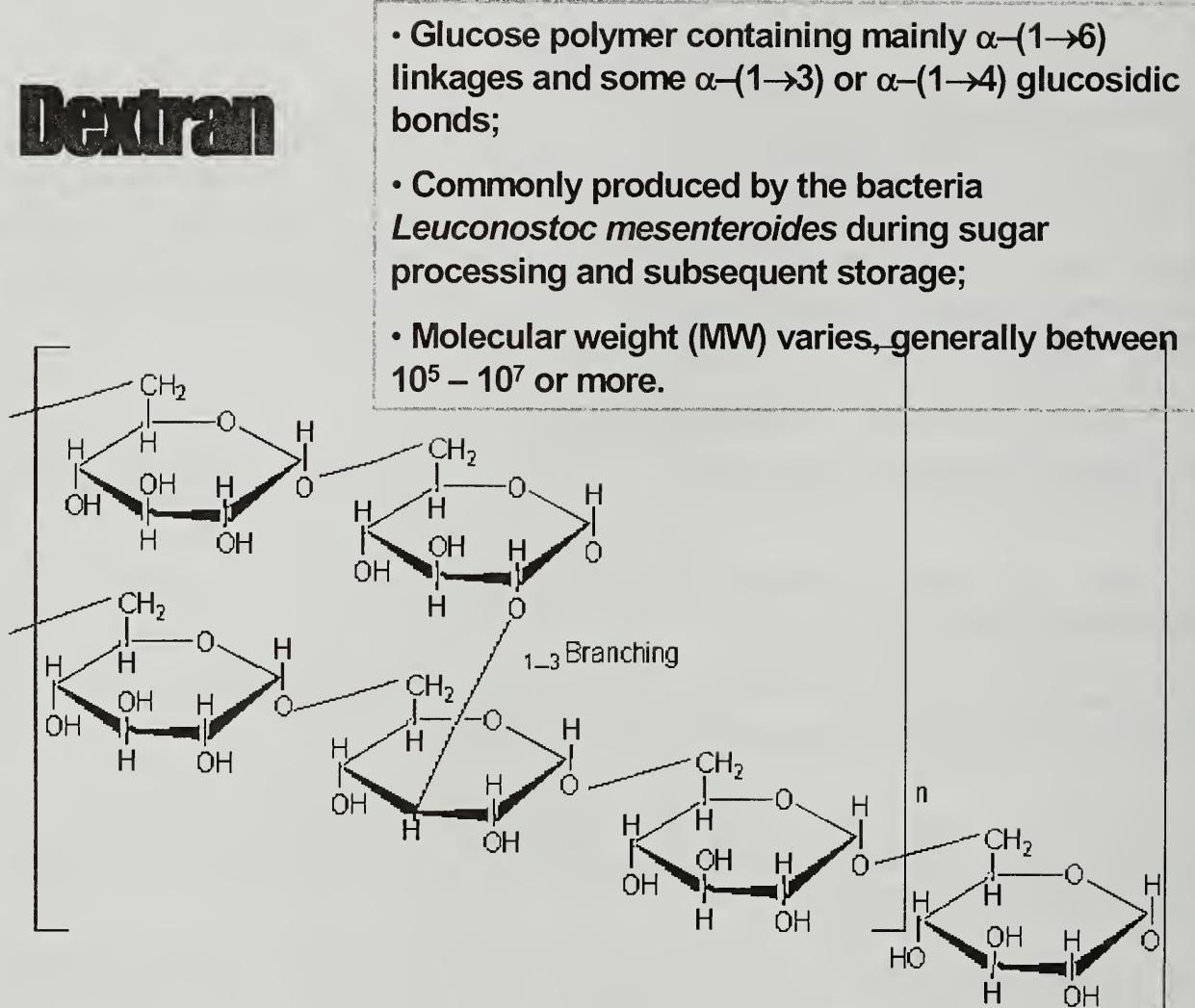
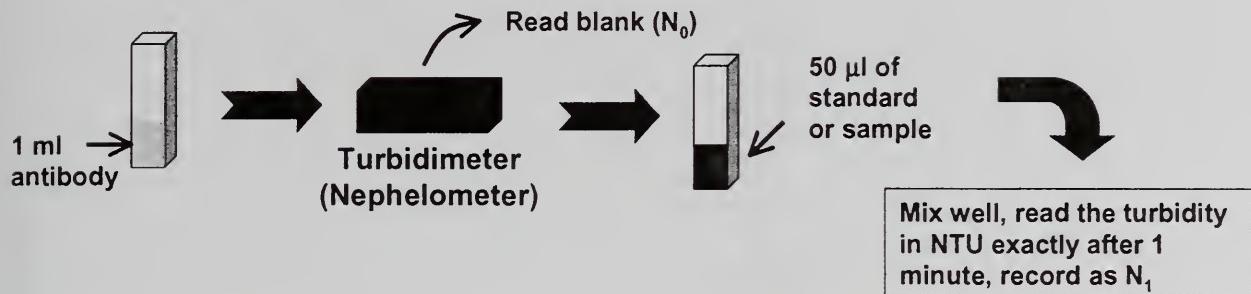


Table 1. Testing methods pros and cons.

Test Method	Sample preparation	Equipment	Disadvantages
Haze (method GS1-15, 1994) [5]	Alpha amylase, TCA ppt.	Nephelometer	Sample preparation time
Robert's (AOAC 988.12) [6]	Precipitate and filter	Spectrophotometer	Hazardous reagent (conc. H ₂ SO ₄)
Antibody Sucrotest [7]	Filter	Nephelometer	Expensive antibody; needs collaboration

Figure 2. Diagram of the Midland Sucrotest procedure.

Test Procedures



Calculate the NTU value of the standard or sample as:

$$N_s = N_1 - N_0$$

Use the N_s of standard solutions to establish standard curve. Calculate the regression equation. Use the N_s of sample solution (sugar or candy) and the regression equation to calculate the dextran concentration in the sample solution.

A new calibration curve was established every time a test was performed. All calibration curves for sample measurement used 2M MW dextran standard.

Table 2.

Dextran content of Refined white sugar from two suppliers

Supplier A

Samples	Dextran (ppm)
	dry wt. Basis
1	74.41
2	52.29
3	212.88
4	147.49
5	142.44
6	111.43
7	59.26
8	23.92
9	60.94
10	48.20
Average	93.33

Supplier B

Lot #	Dextran (ppm)
Samples	dry wt. Basis
1	3.56
2	138.83
3	78.55
4	81.49
5	59.22
6	98.50
7	65.32
8	48.93
9	26.67
10	4.20
11	1.03
12	36.24
13	8.70
14	53.18
Average	50.32

Table 3. Experiment design to test effect of dextran

Run #	Flavor Variable	Dextran (ppm, dwb)
1	A	20
2		250
3		500
4	B	20
5		250
6	C	20
7		250
8	D	20
9		250
10	E	20
11		250
12	F	20
13		250

Table 4. Dextran affects candy B&R

Flavor	Control	250 ppm Dextran	500 ppm Dextran
Flavor A	2.30%	3.40%	4.20%
Flavor B	1.50%	2.90%	
Flavor C	1.20%	1.80%	3.70%
Flavor D	1.70%	2.30%	
Flavor E	1.90%	2.20%	
Flavor F	1.20%	2.70%	

Table 5. Effect of dextran on candy geometry

Sample	Dextran (ppm dwv)	Height (cm)	Diameter (cm)	Weight (g)
Flavor A control	20	9.09	2.05	31.9
Flavor A	500	9.28	2.01	31.5
Flavor C control	20	9.15	2.05	32.2
Flavor C	500	9.39	2.03	32.3

Figure 3. Effect of dextran on height/weight ratio.

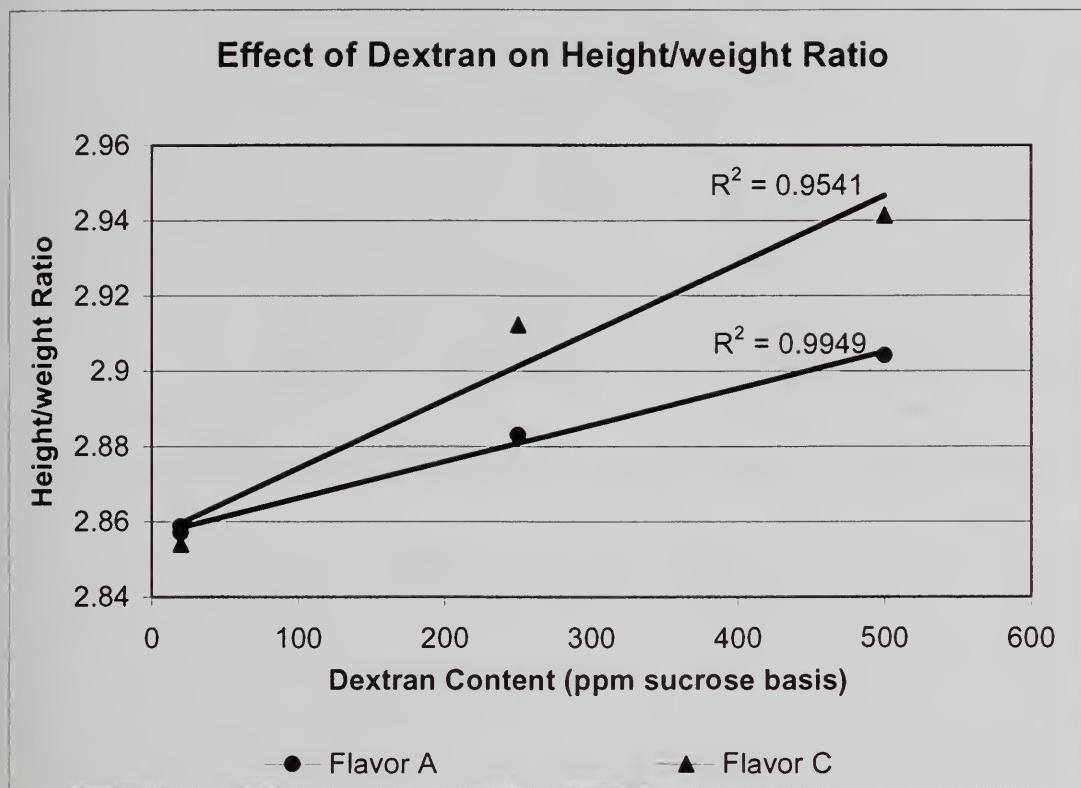


Figure 4. The effect of dextran on package weight

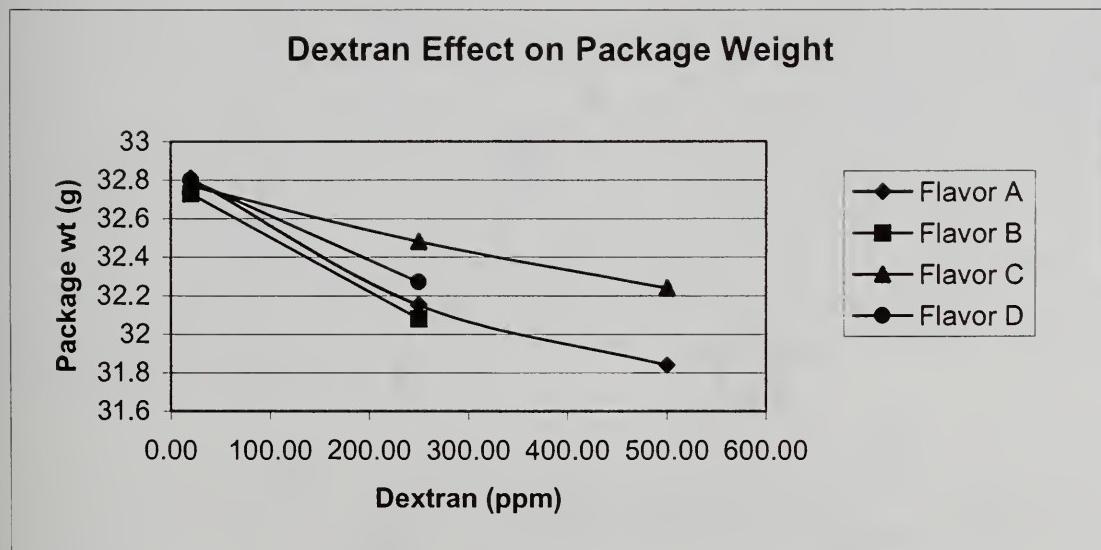


Figure 5. Correlation of dextran in raw vs. refined sugar.

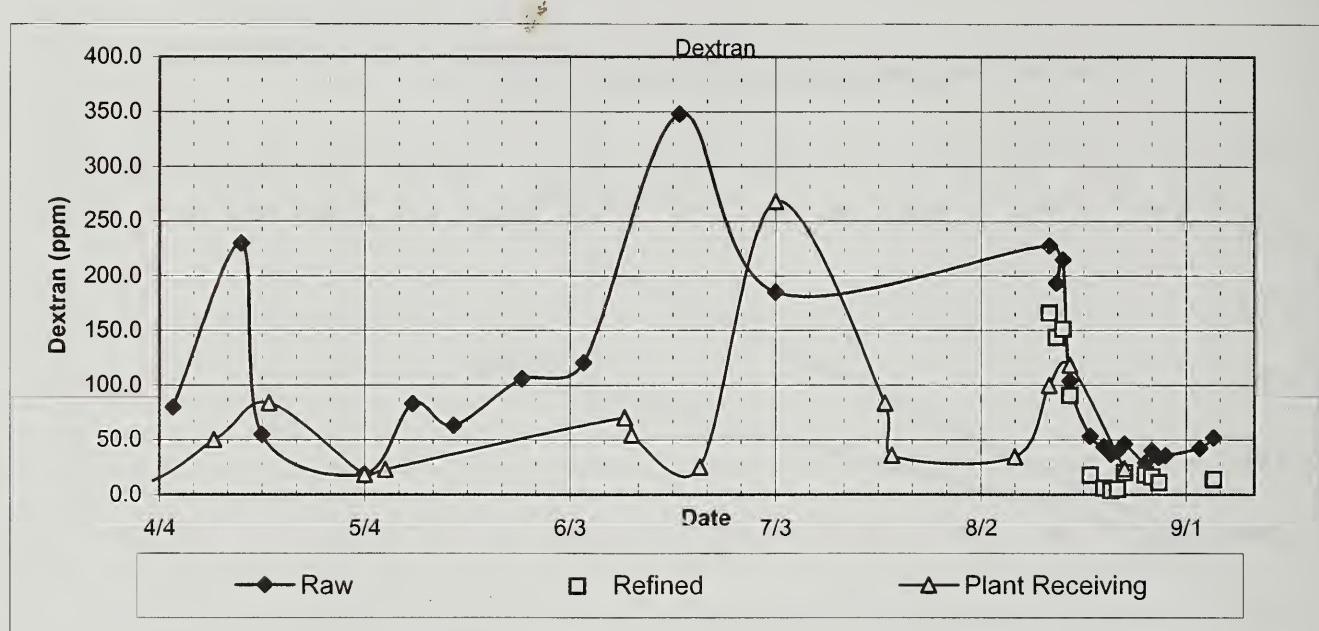
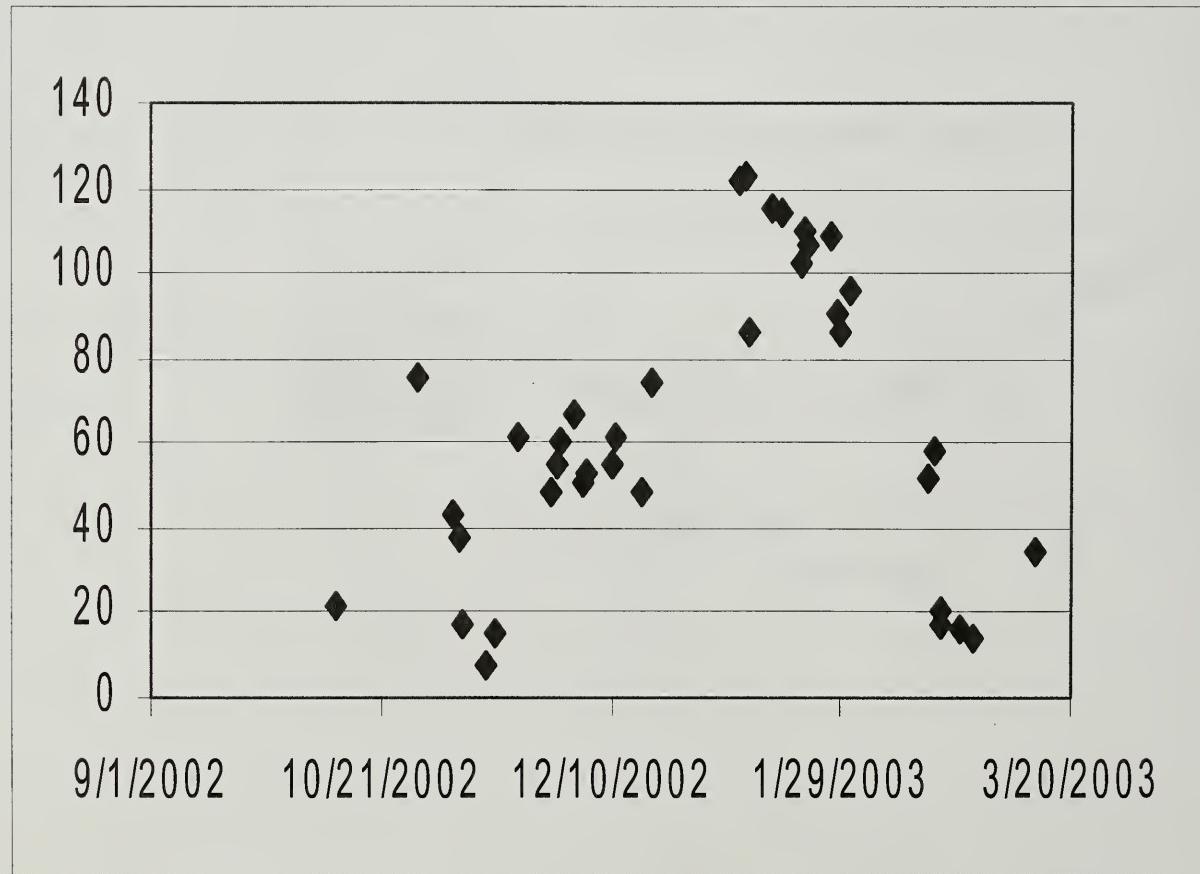


Figure 6. Dextran content in Refined Sugar fluctuates, but is controlled to <125 ppm



DEXTRAN IN WHITE SUGAR: A COMPARISON OF THREE METHODS

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ABSTRACT

The analysis of dextran is important to the sugar industry at all stages. It is well known that dextran is deleterious to the process when present in cane or beet juice, and high dextran raw sugars cause problems in production of refined sugar. It is less obvious that dextran can also be a problem in refined sugar. Dextran in refined sugars is implicated in crystal distortion in hard candies, leading to packaging problems and misshaped candies, such as lifesavers. In this study three methods for dextran measurement were compared -- the ICUMSA haze method for dextran in raw sugar, the Roberts copper method, and the Midland monoclonal antibody (MCA) method. It was necessary to modify the ICUMSA haze method for the lower levels of dextran found in white sugar. All three methods gave comparable results, with the MCA method tending to disagree more often. The study showed the range of dextran found in cane, beet and plantation white sugars. The agreement of results by the three methods is due to the presence of mainly high molecular weight dextran (>50,000 Da) in white sugar, which is equally well detected by all three methods. The modified haze method is provided in an appendix.

INTRODUCTION

Dextran in white sugar has received relatively little attention, although a small body of literature documents the deleterious effect dextran has on hard candy production: The dextran causes elongated crystals (Figure 1) which cause distortion of hard candies so that, for example, lifesavers are no longer round.^(1, 2, 3) High dextran concentration also contributes to the collapse of marshmallows, poor graining of fondant and overboiling of the kettle.⁽²⁾ Refer also to the paper previous to this one in this volume.

Several recent inquiries about the levels of dextran in white sugar and the best method for analysis led us to realize that we did not have a data set for dextran in white sugar, as we had not had a need to analyze it before. We therefore instituted a study to determine the concentration of dextran in white beet, cane and plantation sugars by three methods -- the Roberts method, which is an official AOAC method (Association of Official Analytical Chemists, International), the ICUMSA haze method (official ICUMSA Method GS1-15 1994 official for raw sugar) and the antibody method, developed by the Audubon Sugar Institute and marketed by Midland Research. Midland Research Company generously lent us a kit for the study.



Figure 1. Elongation of raw sugar crystals caused by high dextran content.

MATERIALS AND METHODS

White sugars were requested from sponsoring companies of SPRI as well as taken from the SPRI library of sugars.

Analytical methods used were:

Roberts Copper Method. In this method, all polysaccharides are precipitated with 80% ethanol, and dextran is then isolated from this mixture as a copper-dextran complex. The isolated dextran is measured colorimetrically using phenol-sulfuric acid reagent. This method is an official AOAC method for dextran in raw sugar.

ICUMSA Haze Method for Dextran in Raw Sugar (GS1-15, 1995, Official). In this method, a sugar solution is made up to 50% ethanol and the turbidity produced is measured spectrophotometrically and compared to a standard dextran curve. Prior to ethanol addition, the sugar is treated with amylase to remove interfering starch and trichloroacetic acid (TCA) to remove protein. It was necessary to modify the ICUMSA haze method for white sugar. The modifications are detailed in the next section.

Midland Monoclonal Antibody Method. A specific antibody to dextran is reacted with dextran in a sugar solution to produce turbidity, which is measured with a turbidimeter and referred to a single point calibration.

The modifications of the ICUMSA method are summarized in Appendix A. The haze method for dextran in white sugar is described in Appendix B.

RESULTS AND DISCUSSION

The results of the analyses are shown in Table 1 for beet sugar, Table 2 for refined cane sugar and Table 3 for plantation white sugars and several white sugars of unknown origin.

Table 1. Dextran in beet white sugar

SPRI #	Roberts	Haze (Modified)	Antibody
3	20	20	14
4	14	30	27
5	14	0	90
6	21	30	0
7	23	30	5
115	44	30	54
259	38	30	5
260	35	30	32
261	34	20	22
262	35	15	5
263	34	25	16
264	34	25	11
265	32	30	11
Beet Mean (N=13)	29	24	22

Table 2. Dextran in refined cane sugar

SPRI #	Roberts	Haze (Modified)	Antibody
2	96	87	140
173	42	56	55
238	114	92	81
239	86	107	70
240	97	92	129
241a	137	128	124
245	38	66	80
246	29	35	32
247	51	51	37
248	45	46	43
249	51	40	44
250	44	51	39
251	52	40	34
252	41	46	5
253	52	35	32
254	69	20	0
255	38	30	5
256	36	20	5
258	35	35	22
257	34	20	5
266	75	40	72
267	112	97	194
268	65	35	39
269	69	46	63
Cane mean (N=24)	63	55	56

Table 3. Dextran in plantation white sugars and other white sugars

SPRI #	Roberts	Haze (Modified)	Antibody
Plantation white sugar			
182	39	35	45
183	37	35	28
205	59	20	32
206	52	25	11
Mean	47	29	29
White sugars of unknown type or origin			
241b	49	35	0
243	80	87	96
244	109	107	165
5b	187	231	276
172	143	149	152
Mean	114	122	138

The mean results and ranges for the different types of sugars and methods are summarized in Table 4. These data provide a good idea of expected dextran concentrations in white sugars. The three methods gave similar results. Beet sugar had the lowest dextran, on average. Cane averaged about double that of beet, but also had a wider range, with 6 out of the 24 sugars (25%) having >80 ppm dextran. Plantation sugars (a very small sample) have slightly lower dextran than refined cane sugar because they are usually made from canes that have been harvested by hand, and are clean and fresh. Significantly higher results on two of the plantation sugars tested for the Roberts method than the other two methods indicates that plantation sugars may sometimes have more lower molecular weight dextran. The MCA method showed a wider range than the other methods and disagreed more often (Tables 1-3).

Table 4. Summary of dextran in white sugars by three methods.

Type of sugar	N	Roberts ppm	Haze ppm	MCA ppm	Range (Roberts)	Range (Haze)	Range (MCA)
Beet	13	29	24	22	14 - 44	0 - 30	0 - 90
Cane	24	63	55	56	29 - 137	20 - 128	0 - 194
Plantation	4	47	29	29	37 - 59	20 - 35	11 - 45

Figure 2 shows the correlation between the Roberts method and the new haze method, including all the data from Tables 1-3. There is at least a 92% correspondence between the methods for white sugars.

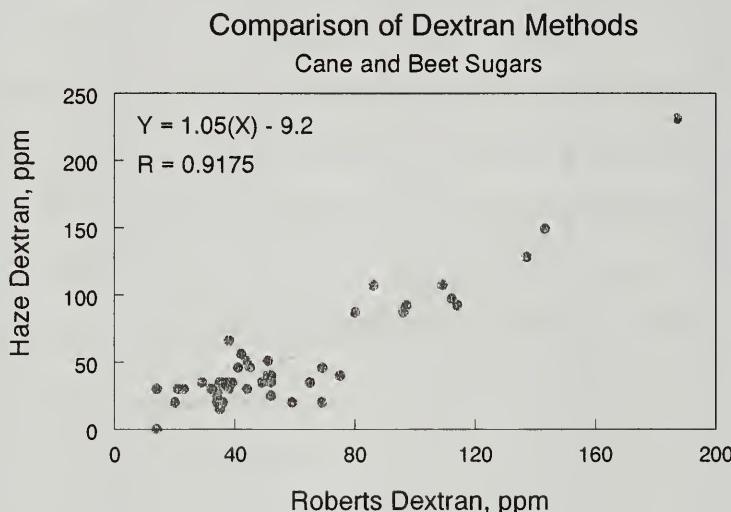


Figure 2. Comparison of dextran in white sugar by the Roberts and haze methods.

CONCLUSION

In conclusion, the Roberts, new Haze and Antibody methods for dextran in white sugar give comparable results. This is attributed to the fact that the dextran in white sugar has a high molecular weight, which is detected by all the methods. Sugars with lower molecular weight dextran, such as some plantation sugars, will show some deviation. The Antibody method was the most rapid and the easiest but is expensive and was more variable. The Roberts method requires a skilled analyst and takes a long time. The new modified haze method is a good compromise and can be used for economical testing of dextran in white sugar.

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Appendix A

Notes on the ICUMSA Haze Method

Examination of the dextran calibration curve of ICUMSA Method GS1-15, "The Determination of Dextran in Raw Sugar by a Modified Alcohol Haze Method," Official 1995, showed that the curve actually consisted of two separate curves, one for lower dextran concentrations (0-80 ppm), having a very shallow slope, and another one for higher dextran concentrations (200-800 ppm, having a considerably steeper slope). This is shown in Figure 1. The authors of the method were aware of this and mentioned it in the method: "The calibration graph should be a gradual curve at low dextran concentration and become almost linear at high dextran concentration."

When we separated the two curves, we found that both were linear, with the lower dextran concentration range having a regression coefficient $R = 1$ and the higher having $R = 0.9987$, but the slopes were different -- 0.0001 for low concentrations and 0.0003 for higher concentrations. When the two lines were combined into one, the regression coefficient was still good, $R = 0.9934$. However, errors would be greater at the lower concentrations, where we were working. The ICUMSA haze calibration curve also has a gap between 80 - 200 ppm dextran.

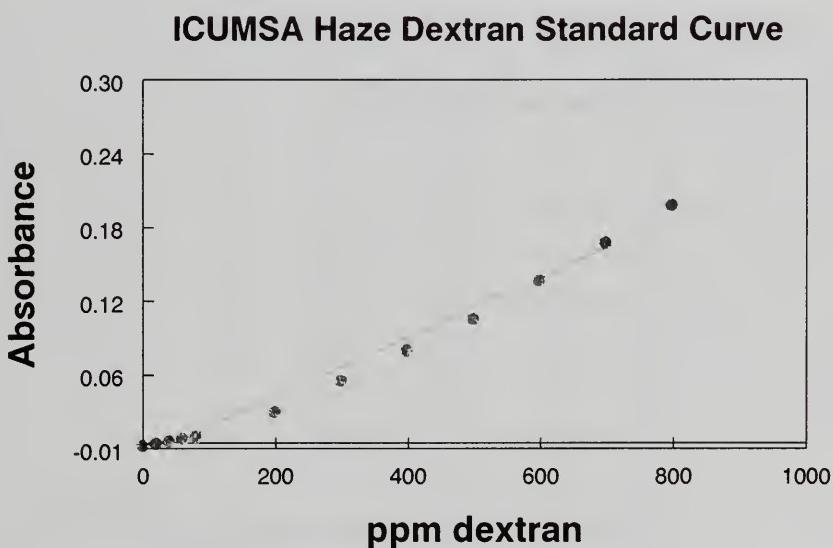


Figure 1. Haze dextran standard curve from the official ICUMSA procedure. The lower and higher dilutions of dextran standards clearly have two different slopes.

We observed large discrepancies between calculated dextran from the standard ICUMSA curve against known dextran for values up to 80 ppm (148% to 7.5%), after which they fell to a more acceptable range. If the two concentration curves were used separately, calculated and actual dextran varied less than 1% in the low range and around 2.3% for the high range. If a curve starting at 80 ppm up to 800 pm was used, the lower value of 80 ppm was off by 47% but was <5% for the rest of the points.

Our goal was to devise a calibration procedure that would produce a single linear regression line through at least 350 ppm dextran. In the ICUMSA procedure, the calibration curve is produced from sucrose containing TCA which is not filtered prior to developing the standard curve, nor is amylase enzyme used. Thus, in the ICUMSA procedure, any starch and protein in the white sugar used for the standard will also produce turbidity upon addition of alcohol, and interfere with the results. This effect will be more pronounced at very low levels of dextran and will give results that are too high.

The new approach to the standard curve was to treat the solutions used to develop the calibration in exactly the same manner as the test samples -- that is, they are treated with amylase, TCA and filtered. We recommend using beet sugar for the standard sugar, if it is available, as beet sugar is typically very low in dextran. If it is not available, a low-dextran cane sugar can be used, as the blanking procedure will help ameliorate interference. The new standard curve is shown in Figure 2.

Table 1 shows the dextran results for 20 whites sugars by both the ICUMSA method and the new method. The new method gave results that are about 12% lower over-all than the ICUMSA method (average of 74 ppm vs 84 ppm). However, there is a 34.6% discrepancy in over-all results for values <80 ppm and only 2.56% discrepancy for values >90 ppm, confirming that results for white sugars will be too high using the ICUMSA method.

Table 1. Dextran in white sugar by different alcohol haze methods.

SPRI #	Type	ICUMSA	New Haze		
5a	Beet	38	15		
3	Beet	42	20		
6	Beet	50	30		
7	Beet	50	30		
115	Beet	50	30		
4	Beet	50	30		
246	Cane	54	35		
241b	?	54	35		
247	Cane	66	51	Mean (11)	
173	Cane	70	56	ICUMSA	New Haze
245	Cane	78	66	55	36
243	?	94	87		
2	Cane	94	87		
238	Cane	98	92		
240	Cane	98	92		
244	?	110	107		
239	Cane	110	107		
241a	Cane	126	128	Mean (9)	
172	Cane	142	149	ICUMSA	New Haze
5b	Cane	207	231	117	120
Mean, all	----	84	74		

Since many raw sugars have dextran values below 350 ppm, twenty raw sugars were analyzed by both methods. Table 2 shows the results using the two calibration procedures. The ICUMSA method gave an average of 154 ppm and the new method gave an average of 162 ppm, an over-all difference of 5.1%.

Table 2. Dextran in raw sugar by different haze methods

SPRI #	ICUMSA	New Haze
1) Col #1	139	169
2) Col #2	209	220
3) Col #3	69	80
4) SM #4	439	390
5) PS 2	64	70
6) #108	54	50
7) #121	239	242
8) #122	114	150
9) #140	49	40
10) #161	214	224
11) A1	194	209
12) A6	199	213
13) C4	64	70
14) E1	119	154
15) E6	114	150
16) F1	134	165
17) F3	304	290
18) F5	59	60
19) F6	49	40
20) F7	261	261
Mean	154	162

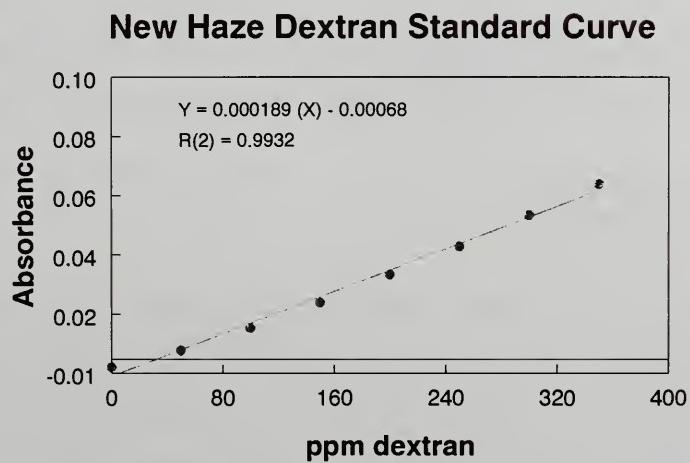


Figure 2. Example of the calibration curve using the new procedure. Beet sugar was used.

Appendix B

SPRI Method for the Determination of Dextran in White Sugar by an Alcohol Haze Method

Introduction.

ICUMSA Method GS1-15 (1994 Official), "The Determination of Dextran in Raw Sugar by a Modified Alcohol Haze Method," has been modified by Sugar Processing Research Institute, for the determination of dextran-like substances in white sugar, both refined cane sugar and white beet sugar. It is also applicable to plantation white sugar.

Definition

Dextran is defined as a high molecular weight, predominantly straight-chained glucose polymer with a majority of 1-6 glucosidic linkages, formed by the action of certain species of bacteria, especially *Leuconostoc mesenteroides*, on sucrose during juice storage and under certain processing conditions. Dextran in white sugar may cause haze in liqueurs and beverages.

Principle

This method measures the haze formed by dextran-like polysaccharides when a solution of sugar is made up to 50% ethanol. Soluble starch is destroyed by incubation with a suitable enzyme. Protein is removed by precipitation with trichloroacetic acid followed by filtration using Celite 545 filter aid. Haze is produced by diluting an aliquot of the treated solution with an equal volume of ethanol. The turbidity of the haze is measured at 720 nm. The method is standardized against commercially available dextran.

REAGENTS

a) Standard dextran. Use Pharmacia DextranT500 or equivalent. Determine the moisture content in duplicate, to 2 decimal places, by drying approximately 2 g of the solid in an oven at 105°C for 3 hours. Record the weights to 0.1 mg. Individual determinations must be within 1 % of the mean of the moisture content. Discard the dried dextran and use only fresh dextran for the calibration curve.

b) Standard dextran solution, 1.0 mg/ml. Quickly weigh a quantity of the undried dextran that contains 0.20 g of anhydrous dextran into a 200 ml beaker, recording the actual mass to 0.1 mg.

i.e., weigh out $\frac{0.20 \times 100g}{100 - \% \text{ water in dextran}}$

Dissolve the dextran by adding 1-2 ml water to form a slurry. Allow the dextran to become uniformly hydrated by standing for about 10 min with occasional stirring. Add more water in small aliquots while a gel mass remains. When about 25 ml has been added and no gel is present, wash the slurry into a 200 ml volumetric flask with water to a volume of about 80 ml. Place the flask in a boiling water bath for 30 min. Cool to room temperature in a cold water bath, then make up to the mark with water. Prepare standard dextran solution daily; do not store overnight.

c) **10% Trichloroacetic acid (TCA) solution.** Dissolve 20.0 ± 0.1 g trichloroacetic acid in distilled water and dilute to 200 ml. This reagent will keep for two weeks, stored under refrigeration in a dark brown bottle.

d) **Absolute ethyl alcohol.**

f) **Standard sucrose.** Use only pure refined beet sugar with a starch concentration of less than 2 mg/kg. (Most beet sugars are negative for starch.)

g) **Starch-removing enzyme.** A heat stable liquid α -amylase, such as Novo Termamyl or 120L Miles Takalite L340.

NOTE - If any other enzyme is used, check that dextran is not attacked by digesting a standard dextran solution with 1 drop of the enzyme at 55 ± 5 °C for 15 min; add TCA, filter and measure dextran haze as for standards. The absorbance should be within 5% of the reading obtained for the same dextran standard without enzyme treatment.

h) **Celite 545 filter aid.** Use as received; no further preparation is needed.

APPARATUS

Spectrophotometer, suitable for the measurement of absorbance at 720 nm.

Water bath, capable of operating at 50-60°C; a gyratory water bath is preferred.

Analytical balance - readable to 0.1 mg.

Stopwatch.

Volumetric flasks - 200 ml, 100 ml, 25 ml.

Funnel - to fit into 100 ml volumetric flask.

Automatic pipetor - 10 ml.

Disposable 1-cm cells.

Buchner funnel and flask - 5.5 cm and 250 ml respectively.

Burettes - 25 ml and 50 ml capacity.

Conical flask - 200 ml.

Beaker - 250 ml.

Filter paper - Whatman No 5, 5.5 cm.

DEXTRAN DETERMINATION IN WHITE SUGAR

1. Weigh out 32.0 ± 0.1 g of sugar, transfer to a 200 ml conical flask, add 50 ml of distilled water, stopper and dissolve using a magnetic stirring plate.
2. Add 0.1 ml starch enzyme. Mix contents well, stopper the flask, and place the flask in a shaking water bath at $55 \pm 5^\circ\text{C}$ for 15 min. Cool the flask to room temperature in a cool water bath.
3. Add 10.0 ml of TCA solution into a 100 ml volumetric flask. Transfer the sugar/enzyme mixture quantitatively to the flask, make up to the mark with water, stopper and mix well.
4. Pour the solution into a dry 250 ml beaker. Add two heaping teaspoons (about 6-8 g) of Celite 545 filter aid and mix well. Filter the mixture through a 5.5 cm Buchner funnel (Whatman No 5 filter paper) under vacuum, discarding the first 10 to 15 ml of filtrate .
5. Using a 25 ml graduated burette, add 12.5 ml of the filtrate to each of two clean, dry 25 ml volumetric flasks.
6. Add alcohol slowly from a 50 ml burette to the 25 ml mark of one flask, gently swirling the flask. The time for the alcohol addition should be 30 - 60 seconds. Mix the contents of the flask by inverting gently three times. Start the stopwatch immediately the mixing step is complete.

NOTE 1 - Add alcohol within 20 min of addition of the TCA solution.

NOTE 2 - A void vigorous shaking of the flask as it may cause coagulation of the dextran haze.

7. To the other flask add distilled water to the 25 ml mark and mix. This is the test blank.
8. 20 min after the completion of the mixing step read and record to 0.001 the absorbance of the test solution against that of the blank solution at 720 nm. Immediately after reading, visually inspect the contents of the test solution cell to check for flocculation. If the haze has flocculated, repeat the analysis.

CALCULATION OF RESULTS

Obtain the dextran concentration in the raw sugar directly from the standard curve by reading against the corresponding solution absorbance. (That is, the dextran result obtained from the standard curve is the result in mg/kg in the tested sugar.) Express results to the nearest mg/kg sugar.

PREPARATION OF STANDARDS AND CALIBRATION GRAPH.

Standard solutions are prepared just as actual samples for testing. In eight 50 ml volumetric flasks prepare the standard solutions as described below.

1. Weigh 32 ± 0.1 g white beet sugar into a 200 ml conical flask
2. Add standard dextran (1mg/ml) solution and water amounts according to Table 1, and mix until the sugar is dissolved.
3. Add 0.1 ml starch enzyme, and mix well.
4. Heat for 15 ± 1 minute in a very slow gyratory water bath at $55 \pm 5^\circ\text{C}$.
5. Cool and proceed from step 3 through step 6 in the previous section for determining dextran in white sugar.
6. Zero the spectrophotometer with the blank sample.
7. 20 min after completing the mixing step, record the absorbance of each test solution.
8. Calculate the actual concentration of dextran in each flask (Table 1) using the moisture in the dextran, and the actual weight taken, e.g.:

Calculation of Actual Dextran Concentration: Example

Moisture of Dextran = 12.2%

Mass of Undried Dextran equal to 0.2 g of

$$\text{Anhydrous Dextran in 200 ml water} = \frac{0.20 \times 100}{100 - 12.2} = 0.2278 \text{ g}$$

Actual Mass of Dextran weighed out = 0.2285 g

$$\text{Actual concentration (350 mg/kg)} = 350 \times \frac{0.2285}{0.2278} = 351.08 \text{ mg/kg (actual)}$$

9. Plot the actual dextran concentration (mg/kg), as shown in the example above for each dextran standard and calculate the curve of best fit.

Table 1. Standard dextran solutions for determining the calibration curve (in 50 ml volumetric flasks).

Flask No.	Sucrose, g	Dextran Conc. (mg/kg of sugar)	ml dextran stock solution (1mg/ml)	Water, ml
1	32	0	0	50
2	32	50	1.6	48.4
3	32	100	3.2	46.9
4	32	150	4.8	45.2
5	32	200	6.4	43.6
6	32	250	8.0	42.0
7	32	300	9.6	40.4
8	32	350	11.2	38.8

HARMONIZATION OF SUCROSE STANDARDS FOR BEVERAGE MANUFACTURERS

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A technical manual on granulated sucrose will be published through the International Society of Beverage Technologists (ISBT). ISBT's objective is to enhance the promotion, development and dissemination of knowledge relating to the Art and Science of Beverage Technology¹. This work is accomplished through twelve individual committees that have been established to cover various phases of the industry. The granulated sucrose manual was created in a subcommittee of the Sweetener Technical Committee. The manual is a collaborative effort between the beverage industry, sugar vendors and sugar experts. The manual contains global quality guidelines, rationale definitions, paragraphs that explain the importance of certain quality parameters, storage conditions and analytical methods (or references). This paper will focus on the global quality guidelines set forth by the sucrose subcommittee.

THE INTERNATIONAL SOCIETY OF BEVERAGE TECHNOLOGISTS

In 1953, ISBT was formed in Washington, D. C. The society was formed to address the technical and scientific aspects of soft drinks and beverages¹. Membership is open to anyone that is engaged in the science, technology or production of soft drinks¹. Membership is also open to suppliers who hold scientific or technical positions. The current membership is approximately one thousand members worldwide.

ISBT has twelve technical committees that were established to cover various phases of the beverage industry. To date, the committees are: Product and Ingredient Quality, Packaging Technology, Global Issues and Technology, Quality, Product and Process Technology, Water Quality and Treatment, Sweetener, Supply Chain, Sanitation and Microbial Control, Environment, Health & Safety, Non-Traditional Beverages and Fountain.

¹ www.bevtech.org

SUCROSE SUB-COMMITTEE

Work on the sucrose standards was carried out by the Sucrose Sub-Committee of the Sweetener Committee. Sub-committees are established on an as-needed basis at the committee's direction. Essentially, sub-committees are working teams that develop guidelines, manuals and other materials to assist the beverage community and members in general. The Sweetener Sub-Committee was established in 2003, with the primary objective to develop voluntary industry guidelines for refined sugar.

The purpose of the Sucrose Sub-Committee was to align on quality standards and methods of analysis for refined granular sucrose (cane and beet) used in the international beverage industry. The mission was to develop an ISBT quality guidelines manual for refined granular sucrose that will be useful to international users and suppliers of granular sucrose. The primary objectives were to create global sugar guidelines, standardize guidelines throughout the beverage industry, standardize sugar methods by aligning with ICUMSA methodology whenever possible, to improve quality and to share best practices.

Approximately twenty-two members volunteered to serve on Sucrose Sub-Committee. The group was cross functional and consisted of members from the sugar industry and beverage industry, and an analytical laboratory also participated. The following companies were represented on the committee: Amylum, Cadbury Schwepps, Cargill Sweeteners, The Coca-Cola Company, Danisco, Imperial Sugar, Pepsi-Cola Company, Silliker and the Sugar Processing Research Institute. British Sugar, Central El Palmar, S.A and Tate and Lyle were not members of the sub-committee, but contributed to either the evaluation of the proposed standards, methods or provided advice on the quality aspects associated with sugar.

The sucrose quality manual will be published in May of 2004, to coincide with the Annual Beverage Technology meeting in Long Beach, California. Quality guidelines were established, other quality considerations were mentioned, analytical methods were recommended and storage and handling guidelines were detailed. Standards were assessed based on their impact to the following criteria:

- **Sensory**
Any attribute that negatively impacts the taste, odor or appearance of a beverage.
- **Process Capability**
Any attribute that defines a key parameter in a controlled process and is an important consideration in the beverage industry.
- **Regulatory**
Any attribute whose limit is set by governing regulatory agencies.

QUALITY STANDARDS

The quality standards that the committee aligned on along with the methods and rationale are in the table on the following page. The notes refer to explanatory paragraphs in the guidelines.

ISBT Quality Standards for Granulated Sucrose

Standard	Guideline	Method	Rationale	Comments
Color	35 IU Beet 45 IU Cane	ICUMSA GS2/3-10	Sensory	See note 1
Ash (conductivity)	0.015% max.	ICUMSA GS2/3-17	Sensory	
Turbidity	20 max.	ISBT Procedure 1	Sensory	See note 2
Taste/Odor	Typical/no objectionable T&O	ISBT Procedure 2	Sensory	
Arsenic	1 mg/kg	ICUMSA GS2/3-23	Regulatory	
POL (assay)	99.8 min.	ICUMSA GS2/3-1	Process Capability	
Copper	1 mg/kg	ICUMSA GS2/3-29	Sensory	
Iron	1 mg/kg	ICUMSA GS2/3/7/8-31	Sensory	
Floc potential	Passes test	ISBT Procedure 3 or ICUMSA GS2/3-40 (A)	Sensory	See note 3
Sediment	10 mg/kg	ICUMSA GS2/3-19	Sensory	
Lead	0.1 mg/kg	ICUMSA GS2/3-24	Regulatory	
Loss on drying /moisture	<0.04%	ICUMSA GS2/1/3-15	Process Capability	See note 4
Microbiological Total mesophilic bacteria	200 CFU/10 g	ISBT Procedure 4	Process Capability	
Yeast and Mold	10 CFU/10 g	ISBT Procedure 5	Process Capability	
Invert Sugar	0.04% max	ICUMSA GS2/3-5(1997)	Regulatory/ Process Capability	See note 5
Sulfur dioxide	6 mg/kg	ICUMSA GS2/1/7-33 or CRA E-67A	Sensory	

OTHER CONSIDERATIONS

The sub-committee discussed other standards that may be considered in the future. Currently, the following standards are being assessed and debated: Quaternary ammonium compounds, processing aids, isovaleric and butyric acids, microbial concerns for non-carbonated beverages and processing residues (pesticides & biocides).

Quaternary compounds can react with caramel in Colas and cause a precipitation reaction. Isovaleric and butyric acids in beet sugar have been linked to off odors in acidified beverages. Non-carbonated beverages need more stringent micro standards due to their higher pH and lack

of carbon dioxide. Biocides used in sugar processing have translated into quality issues in beverage, and it is imperative that sugar be free from pesticide residues.

In addition, new scientific information, test methods and regulations may surface that will require new standards.

ICUMSA

ICUMSA has kindly put together a packet containing the ISBT recommended ICUMSA methods. The ICUMSA Methods for Crystalline White Sugar can be purchased on line at: www.bartens.com

FUTURE WORK

Mary An Godshall and Marie Tanner will present a poster on the turbidity method at ICUMSA (25th session) in an effort to gain it Tentative status. The poster is entitled, "Preliminary Studies on the Reproducibility of a Method to Determine Turbidity in White Sugar". Note: The method did not achieve Tentative ICUMSA status and will be subjected to a collaborative test.

The Sucrose Sub-Committee also plans to develop similar guidelines for liquid sucrose and medium invert sugar. In fact, the committee plans on starting this endeavor in September and welcomes the participation of any interested parties.

CONCLUSION

The collaboration across industries will result in pertinent and higher quality standards, increased global consistency and standardized methods.

DETERMINATION OF COMPONENTS OF INSOLUBLE MATTER IN WHITE SUGAR BY MEANS OF X-RAY MICRO-ANALYZER EDX

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ABSTRACT

This paper presents possibilities for using X-ray micro-analysis (EDX) to determine the components of insoluble matter in white sugar. Solutions of different white sugars from Polish sugar factories were filtered through membrane filters of pore size 8.0 μm (according to ICUMSA Method GS2/3-19). These membranes and retained insoluble matter were analyzed by means of X-ray micro-analyzer EDX. Results showed many different substances were found in the water-insoluble matter of white sugar. The type of the main components of the insoluble matter in sugar is determined by local conditions of technological process in a concrete sugar-factory.

INTRODUCTION

White sugar produced nowadays in Poland usually meets international sugar standards. However, as a result of high competition in the sugar market, many industrial sugar consumers introduce additional requirements. These requirements concern mainly the content of various impurities in sugar and their impact on sugar (sugar solution) properties, such as time of filtration, the content of insoluble matter, creating flocks, turbidity or foaming [2, 3]. One of the main properties of sugar solution is quality of filtration, which depends on the content of water insoluble matter and the type of its main components. For that reason it is important not only to determine the content of insoluble matter but also to determine its main components. The knowledge of the type of insoluble matter in white sugar seems to be important to explain the impact of local conditions of technological process on its content in white sugar and finally on the decrease of the content of insoluble matter in sugar [1, 4].

The paper presents the possibilities of using X-ray micro-analyzer (EDX) to determine the components of insoluble matter in white sugar.

MATERIALS AND METHODS

The materials for this study were three white sugar samples from different Polish sugar factories. In those samples were determined:

- The content of conductivity ash by ICUMSA Method GS2/3-17
- The color of sugar solution by ICUMSA Method GS2/3-10
- The content of water insoluble matter by ICUMSA Method GS2/3-19
- The content of K, Na, Ca, Mg and Fe by means of atomic absorption spectrometry (SOLAAR 969 AAS). The content of the above elements was determined both in water soluble form and in water insoluble form retained on membrane filters after wet mineralization by means of our own method [6]

The membrane filters after filtration of aqueous sugar solutions (according to ICUMSA Method GS 2/3-19) with retained insoluble matter were analyzed by means of X-ray micro-analyzer EDX. Electronic scanning microscopy VEGA 5135 MM TESCAN and X-ray micro analyzer EDX ISIS system LINK 3000 made by Oxford Instruments was used in this work to study the components of water-insoluble matter in white sugar. The X-ray micro-analysis (EDX) is a non-destructive analytical technique which enables determination of the elements and their content on a microscopic surface. The scanning microscope and X-ray spectroscope are connected by this technique and we can observe objects and analyze the chemical content of the surface of objects at the same time [5, 7].

RESULTS AND DISCUSSIONS

The content of conductivity ash and the color of sugar solution in analyzed white sugar samples are shown in Table 1. The samples B and C are standard quality sugars, but sample A is a low quality sugar. Those sugar samples are more different in relation to the content of water insoluble matter. The content of insoluble matter in sample A was the highest and amounted to 20.1 mg/kg. The lowest content of insoluble matter was in sample C — 5.0 mg/kg, in sample B — 9.8 mg/kg.

In those samples we determined the content of the main components of ash such as potassium, sodium, calcium, magnesium and iron. The content of the above elements was determined both in water soluble form and in water insoluble form by means of our own atomic absorption method. [6] The results of this determination are shown in Table 2.

Table 1. The quality of analyzed white sugar samples

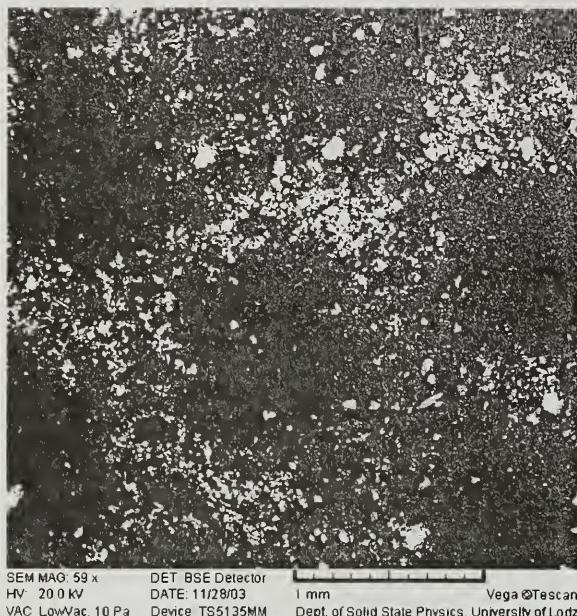
white sugar sample	conductivity ash %	color of sugar solution ICUMSA	insoluble matter mg/kg
A	0.024	45	20.1
B	0.010	21	9.8
C	0.011	28	5.0

Table 2. The content of macro-elements in white sugar samples

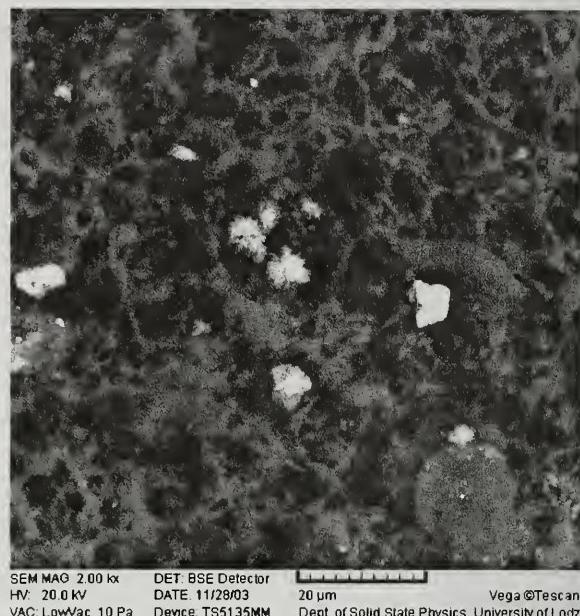
white sugar sample	The content of elements mg/kg							
	K total	Na total	total	Ca insoluble	total	Mg insoluble	total	Fe insoluble
A	26.7	3.6	10.4	3.4	0.1	0.04	1.36	1.19
B	15.1	5.3	3.0	0.7	0.06	0.02	0.15	0.04
C	16.8	3.7	3.9	1.2	0.04	0.02	0.22	0.10

The content of macro-elements in sample A was the highest, particularly in the case of K, Ca and insoluble Fe. The high content of insoluble forms of macro-elements in sample A was correlated with high value of total insoluble matter in this sample. This sugar was characterized by a high amount of insoluble iron, which might be caused by the presence of small pieces of rust. The content of analyzed macro-elements in samples B and C was similar, which corresponds to the similarity of the content of ash in those samples. Therefore, the amount of insoluble matter in sample B which was higher than in sample C is a result of the significant content of organic insoluble impurities in sample B.

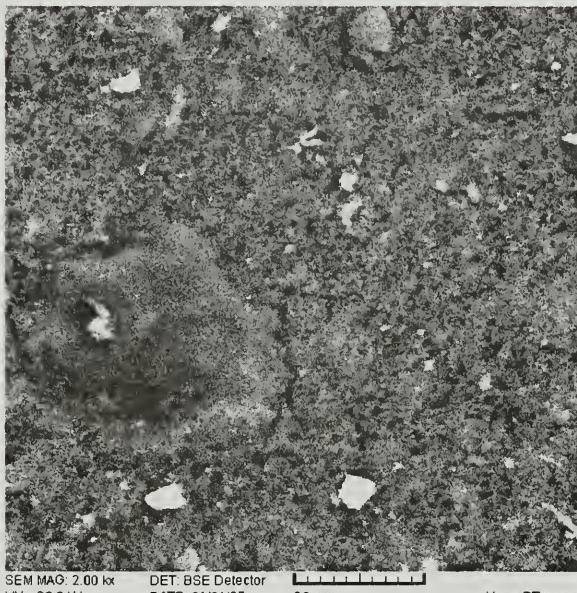
For more precise characterization of insoluble matter in white sugar it is important to identify its main components. We expect that the knowledge of the type of insoluble matter in various white sugars allows to explain the impact of local conditions of technological process on its content in a given white sugar. Sugars from different sugar factories, from different conditions of technological process may contain similar amounts of insoluble matter, but the type of this insoluble matter may be significantly different. The determination of components of insoluble matter in white sugar seems to be the way to decrease its content in sugar. Therefore it is important to find an analytical technique to determine not only the content of elements in insoluble matter but also its type. In this paper, the possibility of using X-ray micro-analyzer (EDX) to determine the components of insoluble matter in white sugar is shown.



sample A (mag. 60 \times)



sample C (mag. 2000 \times)



sample A (mag. 2000 \times)



sample C (mag. 5000 \times)

Figure 1. SEM micrographs of insoluble matter retained on 8.0 μm filters.

The membrane filters with retained insoluble matter from each sugar sample were analyzed by means of X-ray micro-analyzer. During this analysis we can observe the microscopic pictures of surface of the filter with different magnifications. The sample pictures of analyzed sugars are shown in Figure 1.

Using X-ray spectroscopy we can analyze the chemical content of the surface of observed microscopic objects. Three different measurement techniques can be used to determine concentration of elements on the surface. We can measure the content of elements at any point

with diameter about 1.0 μm . In this case we can know the chemical compositions of different objects on the surface. Another possibility is scanning measurement of the whole surface visible in the microscope and collecting data for all the points. In this case we can create a map on which the points with the same X-ray spectrum will have the same color. The third option is the measurement of the average concentration of elements in a selected area. All the above options of measurement can be used during the analysis to identify the components of insoluble matter in sugar.

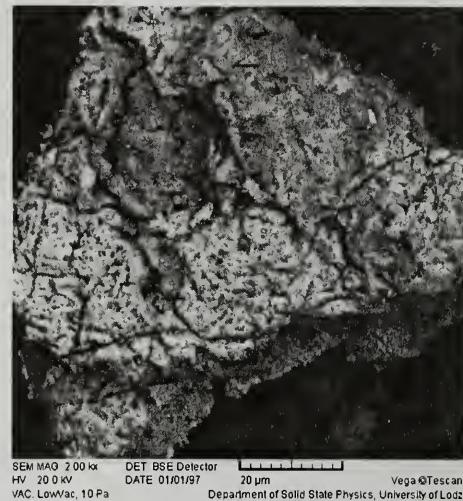
Microscopic pictures of four parts isolated from insoluble matter from sugar sample A and its average chemical compositions are shown in Figure 2. Parts *b*, *c* and *d* contained important amount of iron; however, part *a* contained no iron but only silica. This sugar contained a large amount of insoluble iron (Table 2) and we expected to find in this sugar ferromagnetic impurities such as small pieces of rust [1, 4]. The X-ray microanalysis confirmed the presence of this type of impurity in sugar A — impurities *b* and *c* seem like rust but particle *d* seems like a small piece of stainless steel. We found also a few particles of silica in this sugar — particle *a* in Figure 2.

In sugar sample B we expected to find a significant amount of organic insoluble matter. The retained insoluble matter on filters from sugar B are shown in Figure 3. Many various impurities, of different morphological types, were found in this sugar, the major ones of them are shown in Figure 3. We analyzed the chemical composition of different objects by means of point sampling microanalysis. After comparing the content of the main elements in each object with the morphological image we identified in this sugar the following impurities: cellulose fiber, rust, calcium carbonate, silica, activated carbon (Figure 3). The high content of organic insoluble impurities in this sugar was caused by cellulose fiber and activated carbon, whose high concentration in this sample was detected by means of microanalysis. This result suggests that during production of this sugar there were problems with the filtration process and also with the decolorization process by activated carbon.



Elmt	Spect.	Element	Atomic
	Type	%	%
C K	ED	39.37	48.82
O K	ED	47.51	44.23
Si K	ED	13.13	6.96
Total		100.00	100.00

a)



Elmt	Spect.	Element	Atomic
	Type	%	%
C K	ED	22.95	38.22
O K	ED	37.60	47.01
Si K	ED	1.00	0.71
P K	ED	0.84	0.54
S K	ED	0.22	0.13
Fe K	ED	37.40	13.39
Total		100.00	100.00

b)



Elmt	Spect.	Element	Atomic
	Type	%	%
C K	ED	20.68	29.77
O K	ED	58.44	63.16
Si K	ED	0.94	0.58
P K	ED	1.29	0.72
Fe K	ED	18.64	5.77
Total		100.00	100.00

c)



Elmt	Spect.	Element	Atomic
	Type	%	%
C K	ED	25.79	39.65
O K	ED	42.31	48.84
Si K	ED	1.68	1.11
Mn K	ED	1.14	0.38
Ti K	ED	2.52	0.97
Fe K	ED	25.56	8.45
other	ED	1.00	1.57
Total		100.00	100.00

d)

Figure 2. Macroscopic particles isolated from insoluble matter in sugar sample A.

The cause of the cellulose fibers getting into white sugar is probably low quality of filtration textile. The high content of activated carbon in the analyzed sugar points to a problem with decolorization process, which is caused by low quality of activated carbon or by insufficient filtration after decolorization.



Figure 3. Insoluble impurities isolated from sugar sample B.

Two X-ray maps of different areas from insoluble matter retained from sugar sample C are shown in Figure 4 and Figure 5. Sugar sample C was characterized by standard quality and a low content of insoluble matter — 5.0 mg/kg. No special impurities were found in this sugar. We did X-ray microanalysis of the surface of the filtration membrane with retained insoluble matter from sugar sample C and we created the maps of elements distribution on the filters surface. The main elements beyond carbon and oxygen presented in insoluble matter in this sugar were calcium and silicon. Areas with significant content of iron were also found on this filter. The insoluble matter present in this sugar seems to be a natural residue of lime resulting from the process of purification of juice.

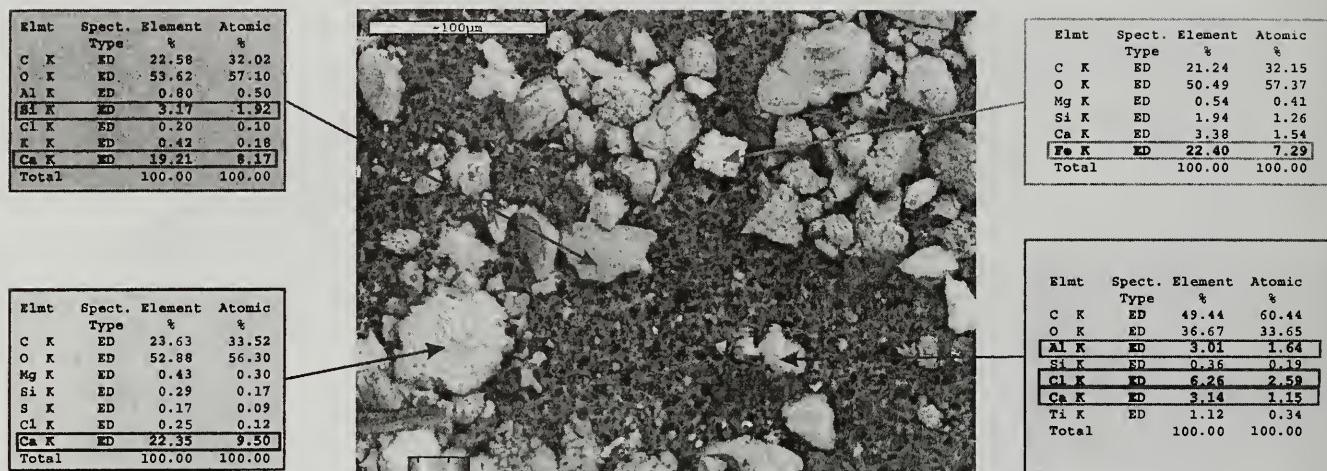


Figure 4. X-ray map from insoluble matter retained from sugar sample C — area 1. The tables present the results of X-ray EDX analysis.

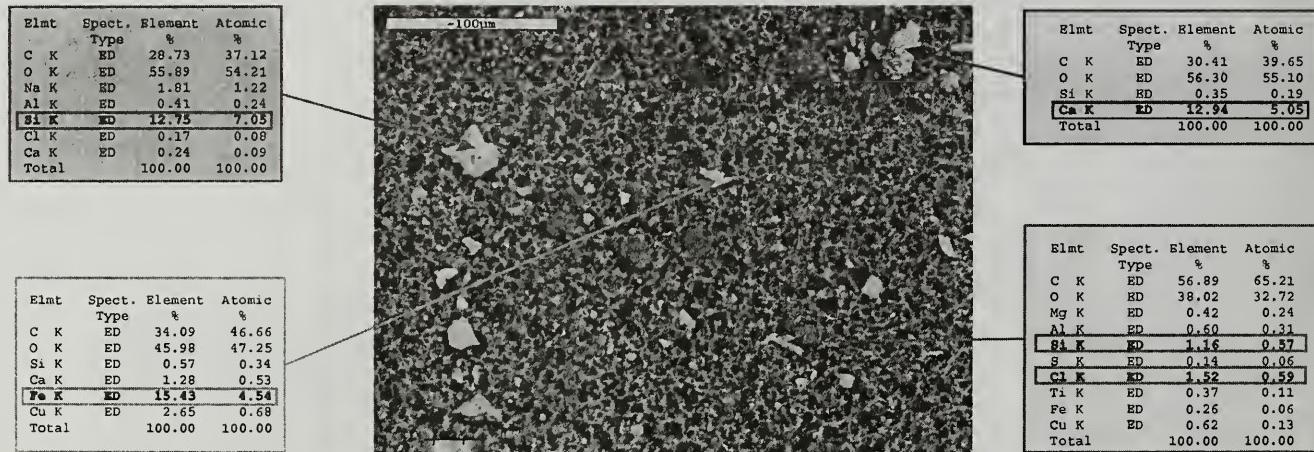


Figure 5. X-ray map from insoluble matter retained from sugar sample C — area 2. Tables present the results of X-ray EDX analysis.

These results confirm the usefulness of X-ray microanalysis for the identification of the type of insoluble matter in white sugar. We are convinced that this technique will prove most useful in finding the causes of the presence of various components of insoluble matter in white sugar produced in different technological conditions.

CONCLUSIONS

- Many different impurities were found in the water-insoluble matter in analyzed white sugars, such as cellulose fiber, carbon, silica, small pieces of iron (rust), calcium carbonate (lime), and others.
- The type of the main component of insoluble matter in sugar is determined by local conditions of technological process in a given sugar factory.

- The proposed technique seems very useful for further study of insoluble matter content in white sugar leading to a decrease of the content of different types of insoluble matter in white sugar.

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THE CONTENT OF SAPONINS IN WHITE SUGAR AND THICK JUICE SAMPLES FROM POLISH SUGAR FACTORIES

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ABSTRACT

The saponin content in white sugar and thick juice from Polish sugar factories was determined by a method based on SPE followed by HPLC. The content of saponins expressed as oleanolic acid monoglucuronide in white sugar from Polish factories amounted to 0.1-0.4 mg of oleanolic acid monoglucuronide in 1 kg of white sugar and the mean value was 0.25 mg of oleanolic acid monoglucuronide in 1 kg of white sugar. The content of saponins in thick juice expressed as oleanolic acid monoglucuronide in thick juices from Polish sugar factories amounted to 100-135 mg of oleanolic acid monoglucuronide in 1 kg of dry substance of thick juices. The mean value was 120 mg of oleanolic acid monoglucuronide in 1 kg of dry substance of thick juices. The free oleanolic acid monoglucuronide was detected in every sample of thick juices. The content of free oleanolic acid monoglucuronide amounted to 3.5- 40 mg of oleanolic acid monoglucuronide in 1 kg of dry substance of thick juices and the mean value was 13 mg oleanolic acid monoglucuronide in 1 kg of dry substance of oleanolic acid monoglucuronide.

INTRODUCTION

Saponins are plant glycosides with strong foaming properties, which cause the characteristic clouding of sugar solutions (floc). Chemically the saponins are triterpenoic or steroic glycosides that undergo disintegration to aglycons (sapogenins) and sugar components (pentoses, hexoses or uronic acids) as a result of exhausting acid, basic or enzymatic hydrolysis [5]. The sites of attachment of sugar components may be one (monodesmosides), two (bidesmosides) or three (tridesmosides)[6]. Sugar beet saponins are triterpeneglycosides. The basic building block is oleanolic acid (Figure 1a) which is glycosidically linked with D-glucuronic acid (Figure 1b) [5].

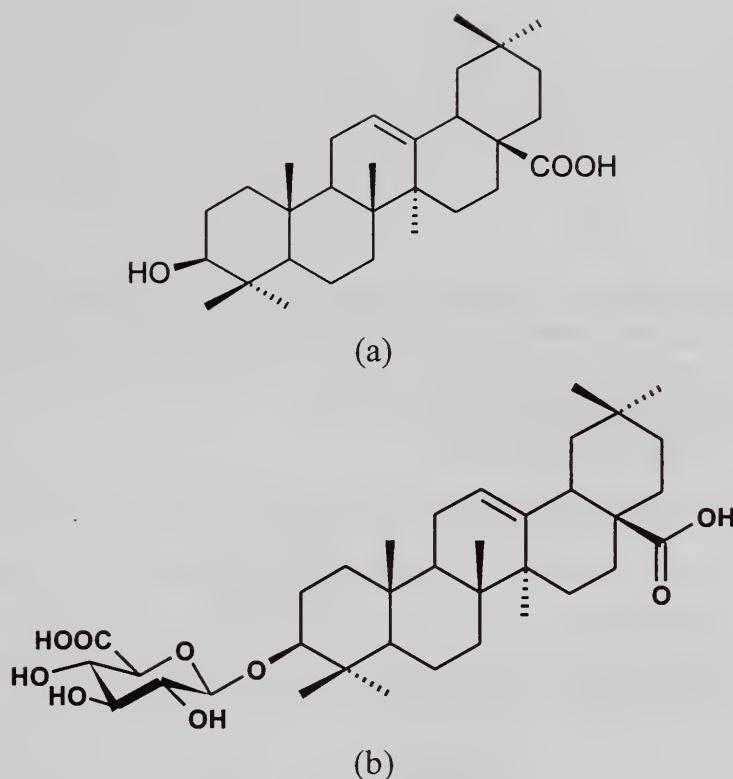


Figure 1. (a) Oleanolic acid (3β)-3hydroxyolean-12-en-28-oic acid ; (b) oleanolic acid monoglucuronide (b).

Several individual saponins such as monodesmosides and bidesmosides from sugar beet and sugar products were isolated and described until now [2,4,8].

Progress in instrumental techniques of analysis, especially the methods of sample preparation and purification together with progress in HPLC techniques makes the determination of saponins in plant material more easy and precise [3] and gives a good background for new procedures of determination of saponins by these methods.

MATERIALS AND METHODS

Materials

- Samples of white sugar (n=9) from Polish sugar factories
- Samples of thick juices (n=9) from Polish sugar factories
- Oleanolic acid – SIGMA
- Freeze dried standard of oleanolic acid monoglucuronide (prepared by HPLC semi-preparative method – our own procedure)
- Potassium dihydrophosphate KH_2PO_4 , analytical grade
- Hydrochloric acid, analytical grade

- Potassium hydroxide, analytical grade
- Acetonitrile for HPLC, gradient grade
- Water R $\geq 8\text{M}\Omega$

Methods:

The method for the determination of saponins from thick juices is based on isolation of saponins from solutions by solid phase extraction (SPE) followed by the HPLC determination of a saponins mixture (Figure 2). The content of the saponin mixture is given in mg of oleanolic acid monoglucuronide in kg of dry substance of thick juice.

The method of saponins determination in white sugar is based on isolation of saponins from white sugar solutions by SPE followed by the HPLC determination of oleanolic acid after hydrolysis (Figure 3). As described by other authors [5], the content of saponins mixture is given in mg of oleanolic acid monoglucuronide in kg of white sugar. The content of monoglucuronide was calculated on the basis of determined oleanolic acid content coming from hydrolysis of saponins mixture (in conditions specified in the procedure [1]).

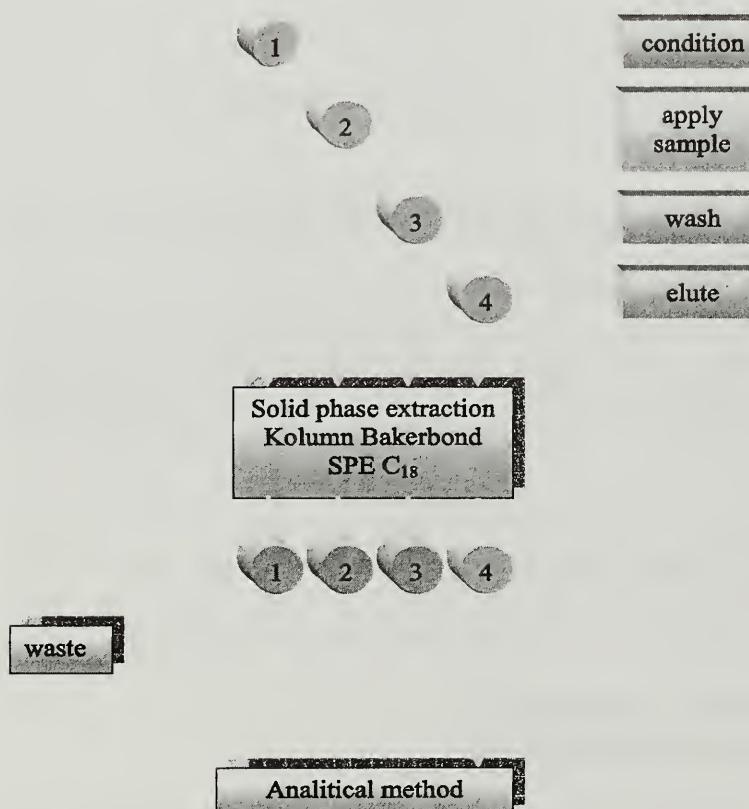


Figure 2. Method for the determination of saponins from thick juices.

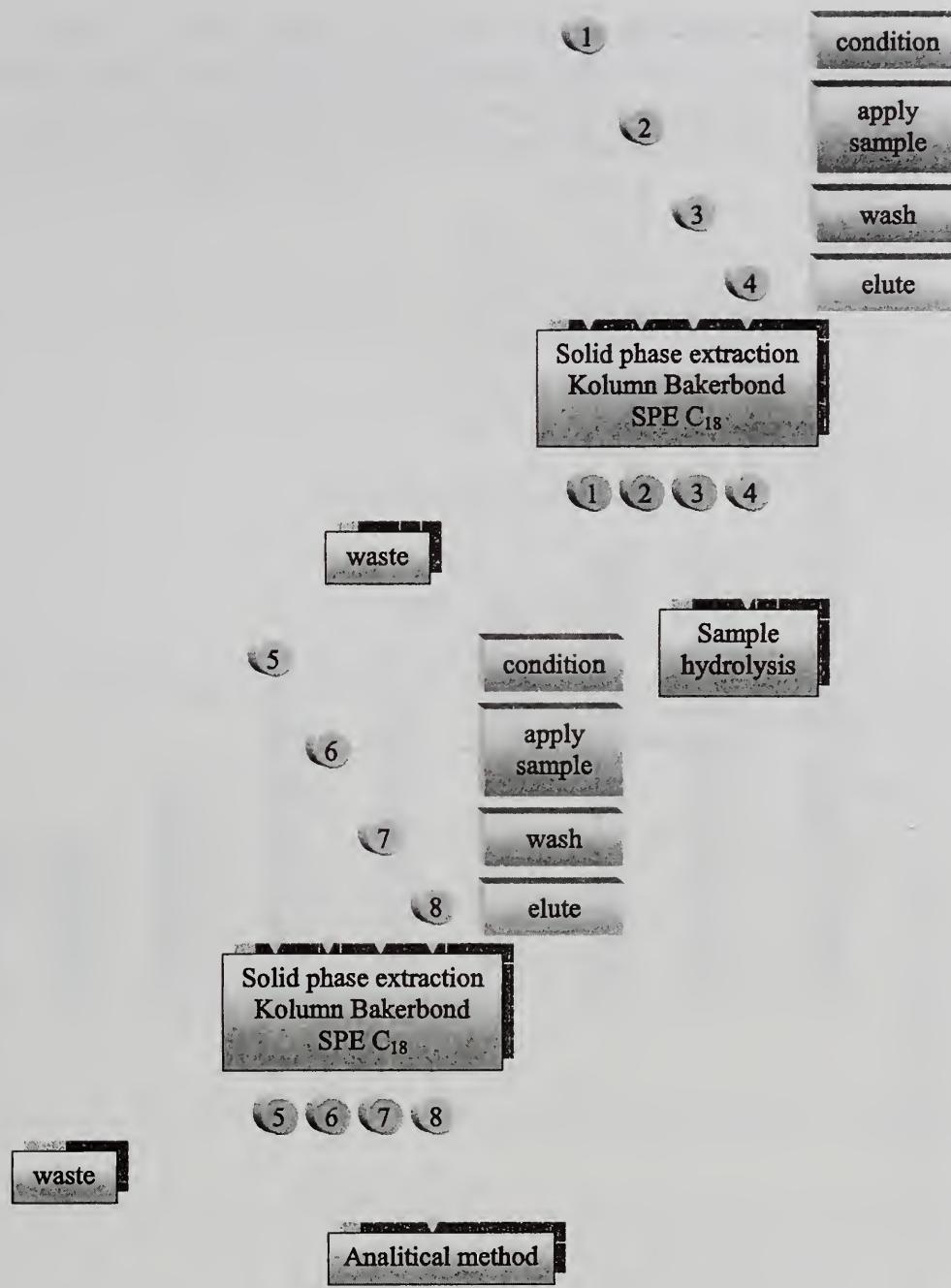


Figure 3 Method for the determination of saponins from white sugar.

HPLC analysis

- analytical column: HPLC Purospher STAR PR-18 endcapped 3 μ m*55
- mobile phases: A 30:70 acetonitrile : 0.005M phosphate buffer
B 90:10 acetonitrile : 0.005M phosphate buffer
- eluent speed: 1 ml/min
- gradient: 1 min – 0% B
3 min – 50% B
5 min – 90% B
7 min – 100% B
10 min – 100% B
12 min - 0% B
- temperature: 30°C
- detector: UV, wavelength 203 nm

RESULTS

The chromatograms of the oleanolic acid standard and the white sugar solution extract (eluate) after acid hydrolysis are shown in Figure 4

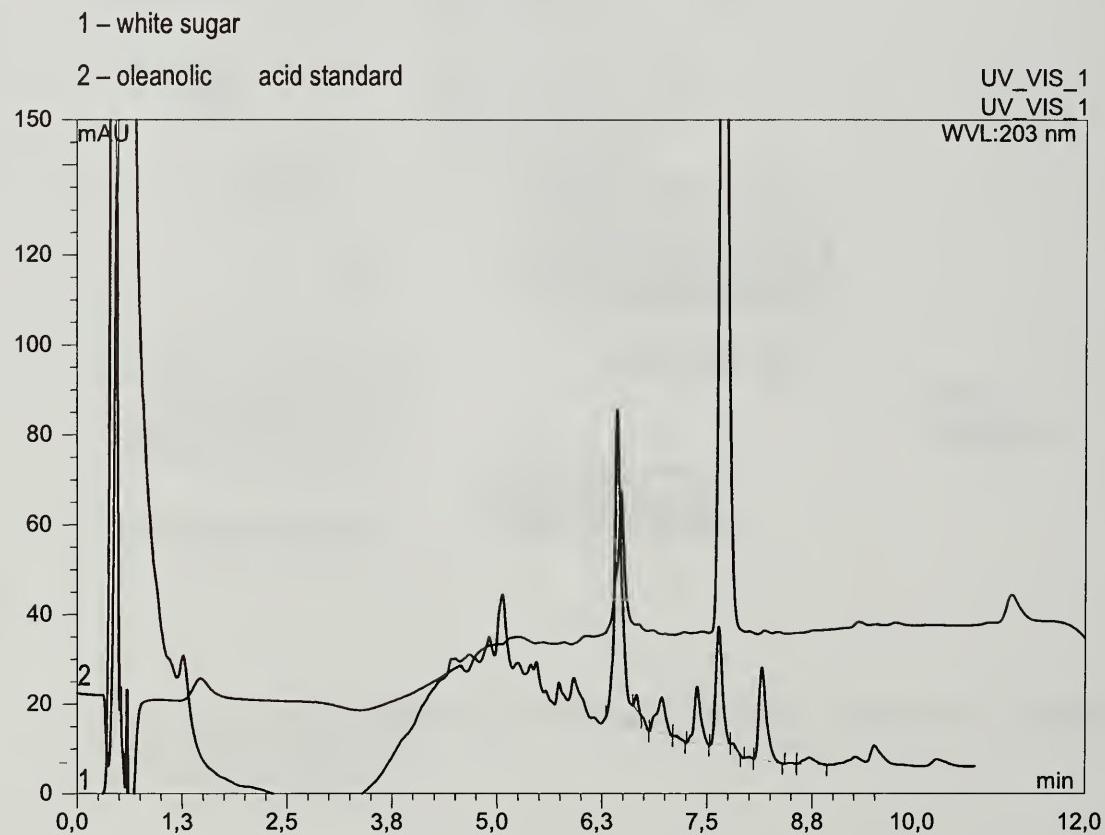


Figure 4. Chromatograms of the oleanolic acid standard and acid-hydrolyzed white sugar solution extracts.

The saponin content in white sugar from Polish sugar factories expressed as oleanolic acid monoglucuronide is shown in Figure 5. The content of saponins expressed as oleanolic acid monoglucuronide in white sugar from Polish sugar factories amounted to 0.1-0.4 mg of oleanolic acid monoglucuronide in 1 kg of white sugar and the mean value was 0.25 mg of oleanolic acid monoglucuronide in 1 kg of white sugar.

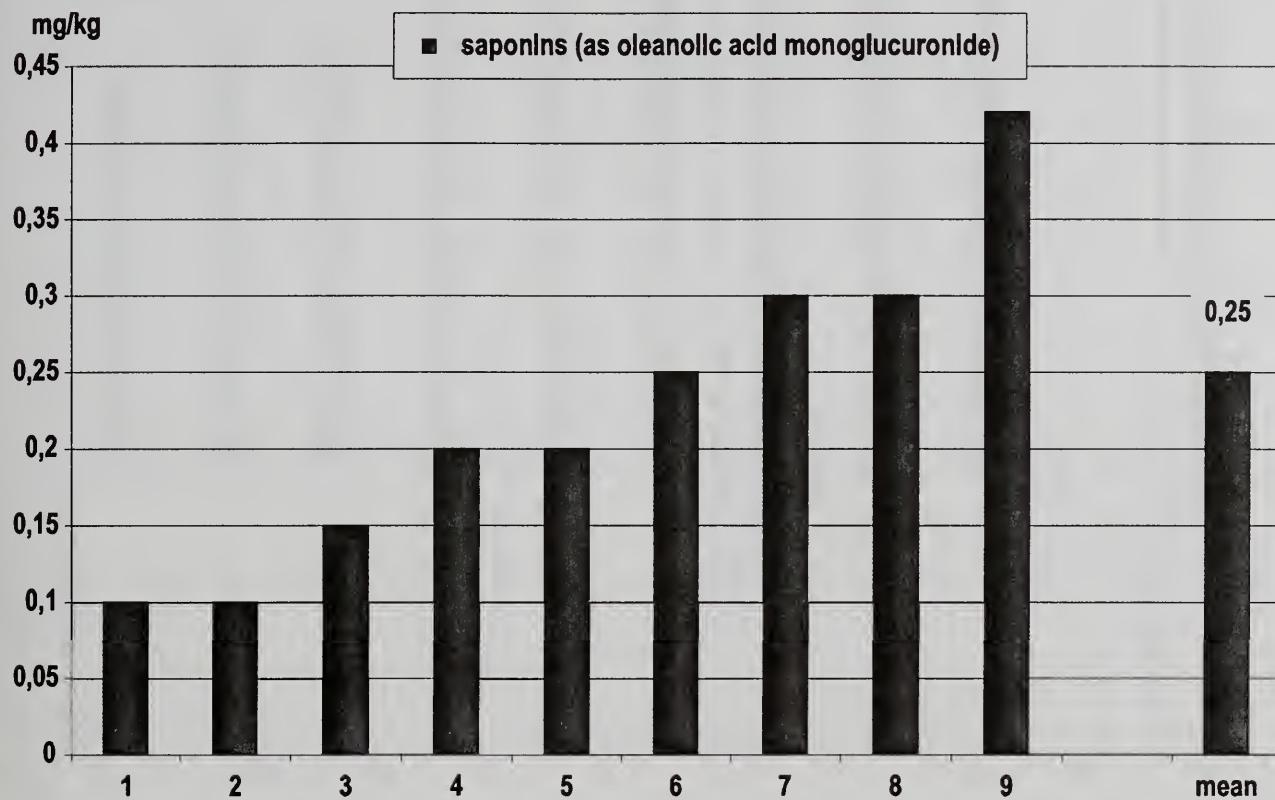


Figure 5. The saponin content in white sugar from Polish sugar factories.

Five sugars out of nine examined contained below 0.2 mg of monoglucuronide. For the reason of saponins content, one can expect the possible usefulness of those sugars for manufacture of soft drinks. According to Van der Poel [7] the limits of saponins content expressed as oleanolic acid glucuronide which result in the foaming of aqueous solutions of sugar are:

< 0.2 mg of oleanolic acid monoglucuronide / 1 kg of white sugar – no foaming
 > 0.4 mg of oleanolic acid monoglucuronide / 1 kg of white sugar – foaming always occurs

The chromatograms of oleanolic acid monoglucuronide standard and thick juices solution extract are shown in Figure 6.

Gradient HPLC makes it possible to detect some saponins (Figure 6). The retention time from 3.8 to 5.3 minutes suits the range of saponins, determined by the standard of saponins from raw sugar beet juice. The signal of oleanolic acid monoglucuronide of retention time 5.2 minutes was detected in every sample of thick juices. It was found that the signals of oleanolic acid

monoglucuronide and saponins having the retention time 4.0-5.2 min are similar and for this reason the sum of saponins is expressed as oleanolic acid monoglucuronide.

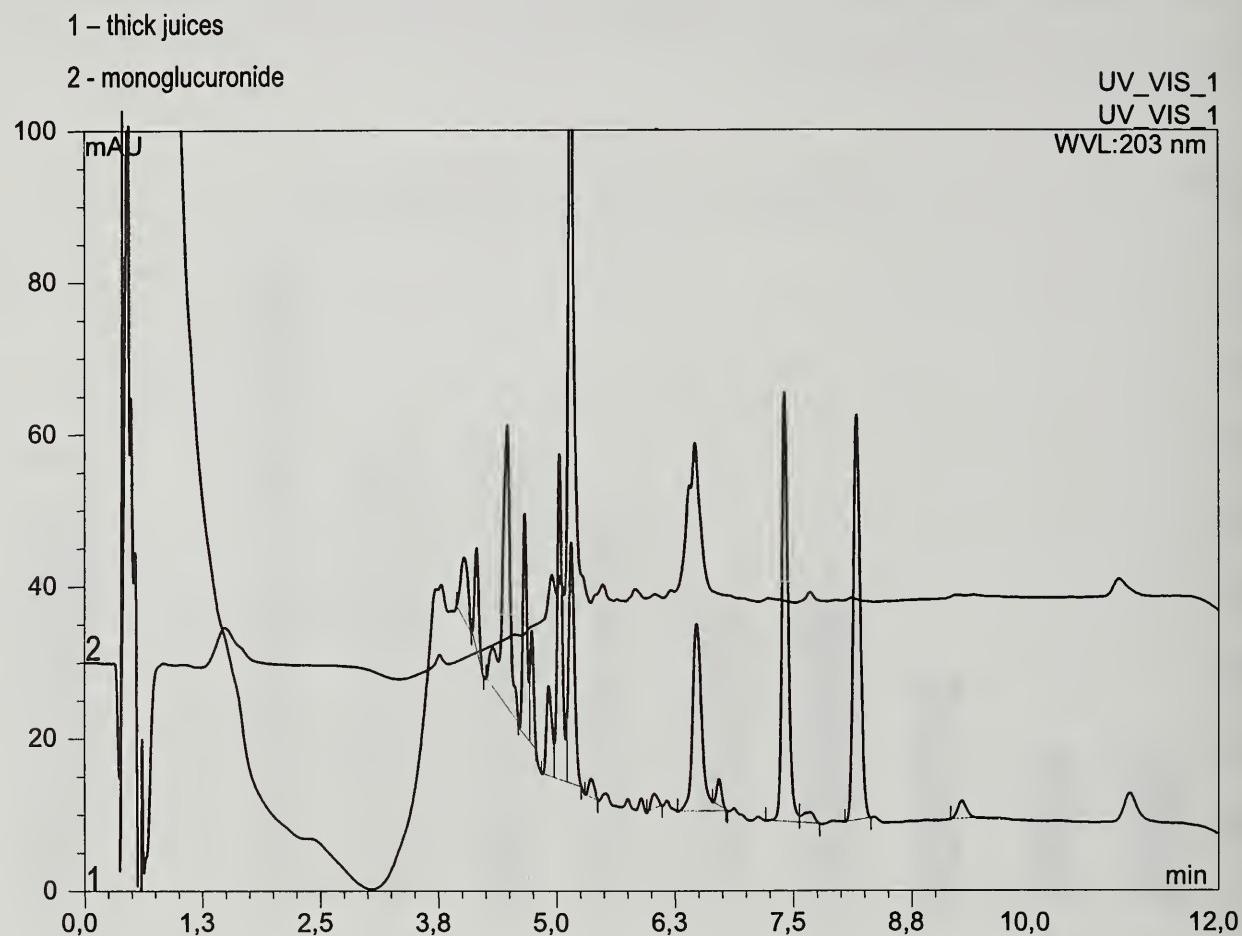


Figure 6. Chromatograms of oleanolic acid monoglucuronide and thick juices solution extract.

The average saponins expressed as oleanolic acid monoglucuronide and free oleanolic acid monoglucuronide content in thick juices from Polish factories is shown in Figure 7.

The content of saponins in thick juices (Figure 7) expressed as oleanolic acid monoglucuronide in thick juices from Polish sugar factories amounted to 100-135 mg oleanolic acid monoglucuronide in 1 kg of dry substance of thick juice and the mean value was 120 mg of oleanolic acid monoglucuronide in 1 kg of dry substance of thick juice. The free oleanolic acid monoglucuronide was detected in every sample of thick juices. The content of free oleanolic acid monoglucuronide amounted to 3.5- 40 mg oleanolic acid monoglucuronide in 1 kg of dry substance of thick juices and the mean value was 13 mg of oleanolic acid monoglucuronide in 1 kg of dry substance of oleanolic acid monoglucuronide. The results obtained for the free oleanolic acid monoglucuronide in the examined thick juices showed diversity in these thick juices. It seems probable that the content of free oleanolic acid monoglucuronide in thick juices depends on technological conditions.

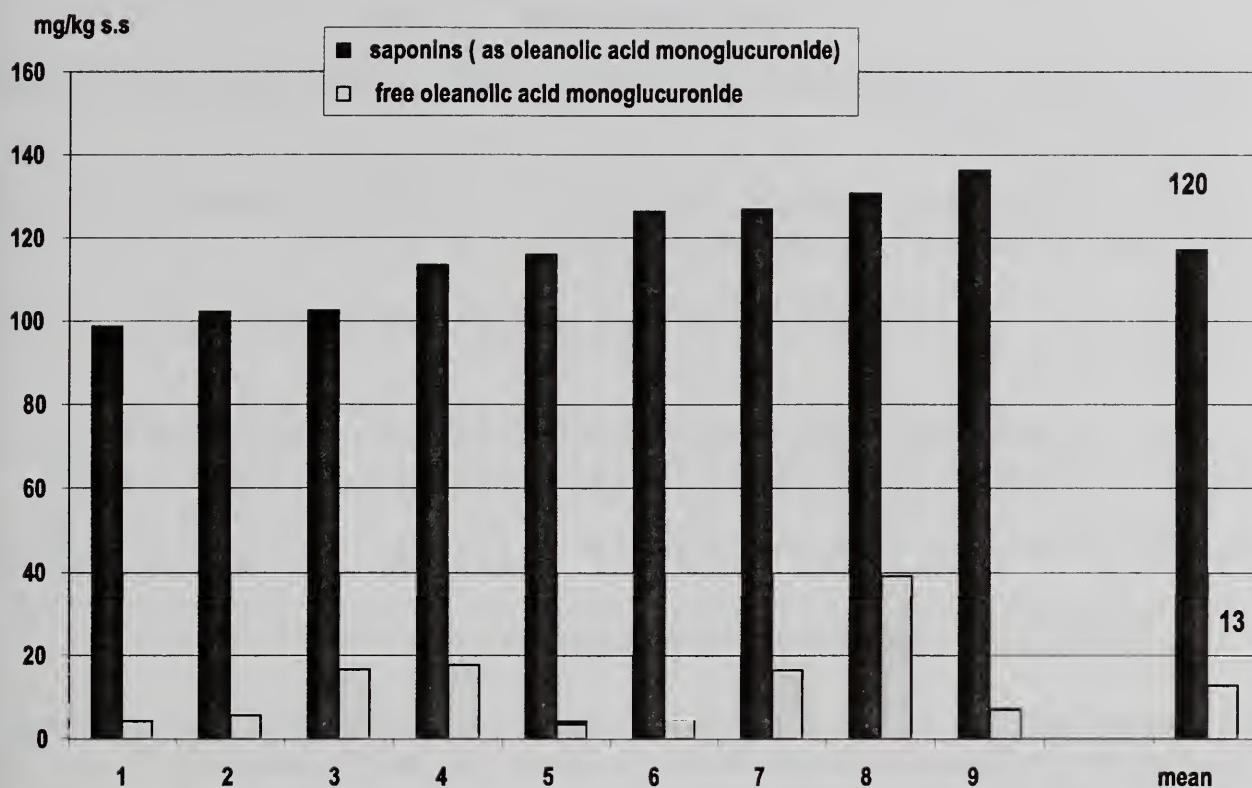


Figure 7. Content of saponins and oleanolic acid monoglucuronide in thick juices from Polish sugar factories.

CONCLUSIONS

- The method of determining saponins based on isolation of saponins from solutions by SPE followed by HPLC determination gives good results in the analysis of saponins of sugar products.
- The content of saponins such as oleanolic acid monoglucuronide in white sugar from Polish factories amounted to 0.1-0.4 mg of oleanolic acid monoglucuronide in 1 kg of white sugar and the mean value was 0.25 mg of oleanolic acid monoglucuronide in 1 kg of white sugar.
- The content of saponins in thick juices expressed as oleanolic acid monoglucuronide in thick juices from Polish sugar factories amounted to 100-135 mg of oleanolic acid monoglucuronide in 1 kg of dry substance of thick juices and the mean value was 120 mg of oleanolic acid monoglucuronide in 1 kg of dry substance of thick juices.
- The content of free oleanolic acid monoglucuronide amounted to 3.5- 40 mg of oleanolic acid monoglucuronide in 1 kg of dry substance of thick juices and the mean value was 13 mg of oleanolic acid monoglucuronide in 1 kg of dry substance of oleanolic acid monoglucuronide.

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COMPARISON OF TWO METHODS OF VOLATILE ANALYSIS FOR DETERMINING THE CAUSES OF OFF-ODORS IN WHITE BEET SUGARS -- SPME AND HEADSPACE

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ABSTRACT

White beet sugars periodically have off-odors, causing them to be rejected by customers. An understanding of the nature and source of the compounds responsible will help in eventually eradicating the problems that cause them. However, determining volatile substances in white sugar is challenging because the amounts present are very small, often in the parts-per-million or even parts-per-billion range. In this study, we describe a set of white beet sugar samples that were received from several locations. Each sugar was given an over-all sensory rating of: 1 = acceptable; 2 = borderline; or 3 = reject, by a sensory panel. The samples were analyzed by two methods of volatile analysis: Solid Phase Micro Extraction (SPME) and headspace analysis. Sample chromatograms were evaluated for compounds at mass to charge ratio (m/z) 60, where volatile fatty acids are found, with the exception of propionic acid. Representative chromatograms illustrating acceptable, borderline, and reject sugars are shown. Samples in the acceptable and borderline categories had lower levels of the volatile fatty acids than did the reject sugars. This was true for both SPME and Headspace. However, it was apparent that SPME was a better technique for volatile analysis.

INTRODUCTION

White beet sugars sometimes have off-odors, which not only affect the value of the sugar, but can also result in their rejection by customers, who do not wish to use a malodorous sugar in their product. Among the causes of off-odors are microbial infection, poor beet quality, beet deterioration in storage, improper beet washing, odorants in centrifugal wash water, mother liquor in crystals and acid or alkaline degradation of sugar in processing.

There has been longstanding interest in understanding and correcting the source of these odors, but only in recent years has technology become available that allows for the rapid analysis of trace volatiles in a matrix such as white sugar. Marsili, et al., (1994) and Godshall, et al., (1995) identified some of the major components responsible for off-odors, using purge and trap procedures and direct thermal desorption. Although many compounds were identified that contributed to the odor of beet sugar, including pleasant odors and off-odors, the major off-odor compounds in crystalline beet sugar were found to be volatile fatty acids (VFA), in particular, butanoic and isovaleric acids. Pihlgard, et al., (1998, 1999, 2000) combined sensory analysis and headspace analysis to identify volatiles from liquid beet sugars of different purities, and followed the progress of volatiles through processing. Pyrazines, furans and aldehydes were found.

Colonna, et al., (1996) examined factory processing streams, wash water and white sugars using purge and trap and various solid phase adsorbents with solvent desorption. Many volatiles responsible for off-odors were identified in the wash water and condensate, which could be a possible source of contamination. Recently, Batista, et al., (2002) reported on optimization of volatile analysis for semi-quantitative analysis of butanoic acid in cane and beet sugars using solid phase microextraction. It is of interest to note that Marsilli (1994) reported the presence of geosmin in white sugars but no other researchers have found it, although it has been found in wash water (Colonna et al., 1996) and beet peels (Godshall, unreported results) and early in the process but not later (Pihlgard, et al., 2000). Geosmin is responsible for the earthy odor of sugarbeets.

In this study, two methods for determining the volatiles in white beet sugar were compared. The first method was SPME, an acronym for Solid Phase Micro Extraction. This is a recent technique that utilizes a fiber coated with an adsorbent material that is placed in the headspace of a sample for a specific period of time and temperature. During this time, the volatiles in the headspace are concentrated on the thin film of the fiber. The fiber is subsequently desorbed with heat into a gas chromatograph-mass spectrometer (GC-MS) for separation and identification of the volatiles.

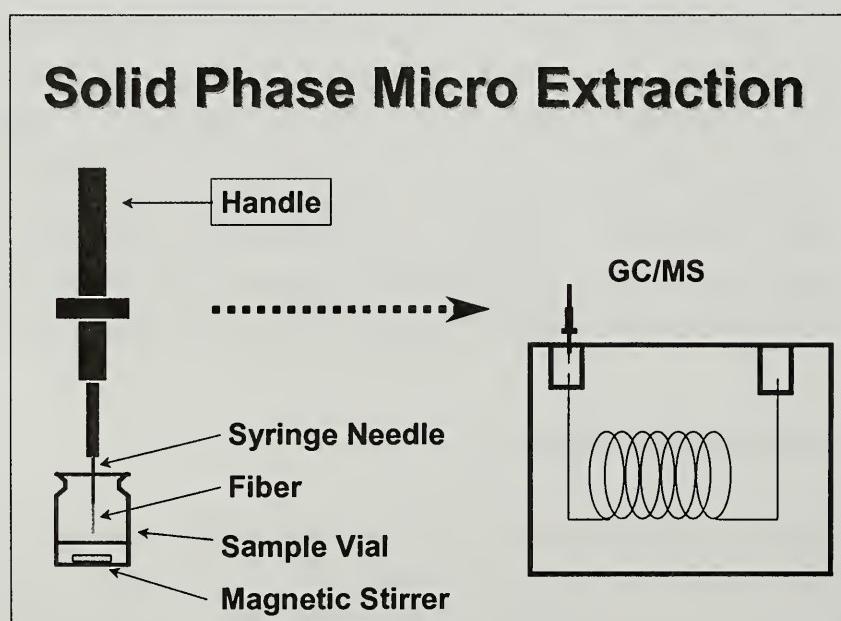


Figure 1 shows a simplified diagram of a SPME apparatus. The second method studied was headspace analysis, which consists of removing a measured volume of the headspace from a sample of sugar and introducing it directly into the GC-MS for sample analysis and data collection. Both methods are simple, inexpensive, rapid, and do not use solvent extraction. Only small amounts of sugar are needed for either analysis and no sample preparation is required.

Figure 1. Diagram of SPME extraction apparatus.

EXPERIMENTAL

A set of white beet sugars was received from seven different locations, with four to seven sugars from each location. Each sample was sensory scored by a SPRI sensory panel, looking at a set of six odor categories. Each sugar was then given an over-all sensory rating as follows: 1 = acceptable; 2 = borderline; 3 = reject. The SPRI results were compared to those of a professional tester from a large confectionery company, and it was noted that SPRI testers tended to be stricter in some instances than the professional tester, rating more sugars as borderline.

The six odor categories were:

1. Sweet aromatic (like flowers or sweet spices)
2. Caramel, browned, chocolate, nutty
3. Barnyard, slightly ammoniacal, meaty
4. Mushroom-like, earthy, beety
5. Sour, fermented, volatile fatty acids, butanoic/isovaleric acids
6. Green

Experimental – SPME

For SPME analysis, approximately 0.75 g of each sugar was weighed into 2-ml vials. Sample vials were placed in a CTC SPME Autosampler (Leap Technologies, Carrboro, NC) and preheated at 65°C for 15 min. The headspace was extracted for 15 min using a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS - 50/30 µm film) fiber (Supelco, Bellefonte, PA).

Experimental – Headspace Analysis

For headspace analysis, approximately 5 g of each sugar sample was weighed into 10-ml vials. The samples were placed in the autosampler, heated at 65 °C and agitated for 20 min. The vials were then punctured and a measured volume of 2.5 ml of headspace was taken and introduced directly into the injection port of the GC-MS.

GC-MS Conditions

Sample volatiles were desorbed from the SPME fiber for 2 min at 270°C into the injection port of an Agilent 6890 GC equipped with a 5973 MS system (Agilent Technologies, Palo Alto, CA). Helium was utilized as the carrier gas under a constant flow of 36 cm/s through a 30 m, 0.25 µm, DB-5 capillary column (J & W Scientific, Folsom, CA). An initial GC temperature of 50°C was held for 1 minute. The temperature was then increased at 5°C/min to 100°C, then at 15°C/min to 270°C and held 5.67 min.

The mass spectrometer was operated in scan mode from m/z 45 to m/z 350 employing 70 eV electron ionization. Compounds were identified using the Wiley mass spectral library (7th Edition).

The total ion chromatogram (TIC) for each sample was run for both techniques, but this study focused on those compounds with a mass to charge ratio of 60, characteristic of volatile fatty acids, with the exception of propionic acid. Each sample was run in duplicate.

RESULTS AND DISCUSSION

Good profiles of the volatile fatty acids (VFA) were obtained using m/z 60 detection. The total ion chromatogram showed lower levels of other compounds, and many of the compounds previously identified in beet sugar were not picked up. This was felt to be due to the small sample size of 0.75 g for SPME. The normal range of white beet sugar VFA (m/z 60) was observed in most of the sugars. These included acetic, butanoic, isovaleric (3-methyl butanoic acid), pentanoic, hexanoic, heptanoic, octanoic and nonanoic acids. Reject sugars did not appear to contain very much of the higher molecular weight volatile fatty acids (heptanoic, octanoic, nonanoic). This was probably due to the very high levels of acetic, butanoic, and isovaleric acids in those samples which had a saturating effect on the fiber used. The odors associated with the VFA identified in the sugars are shown in Table 1.

Table 1. Volatile fatty acids identified in beet sugar and type of odor. (From Fenaroli's Handbook (Furia and Bellanca, 1975), Sigma-Aldrich (2003), and personal observation.)

Volatile fatty acid (Common name)	Type of odor
Acetic acid	Vinegar, sour, acetic
Butanoic acid (Butyric acid)	Rancid, sour, cheesy
Isovaleric acid (3-Methylbutyric acid)	Rancid, cheesy, sweaty
Pentanoic acid (Valeric acid)	Sweaty, rancid
Hexanoic acid (n-Caproic acid)	Sweaty, rancid, sour, cheesy, fatty
Heptanoic acid	Disagreeable, rancid, tallow-like
Octanoic acid (Caprylic acid)	Slightly unpleasant rancid, fruity-acid, oily
Nonanoic acid (Pelargonic acid)	Fatty, cheesy, waxy

A representative sample of the SPME and headspace chromatograms of an acceptable sugar is shown in Figure 2. The abundance measurements shown on the y-axis are relative measurements of peak intensity and not quantitative measurements. The SPME chromatogram (upper left) showed very good integration for the fatty acid peaks and high abundance readings ranging from under 20,000 to about 140,000. In contrast, the same sugar run by headspace analysis (upper right) had much lower abundance ranges from under 2,000 to a maximum of only about 10,000. There is also a lot of noise along the baseline, which is common when dealing with lower concentrations of compounds. Note also that acetic acid was not present on the headspace chromatograms. MS data was not acquired during the time that acetic acid eluted from the column because it coincided with the elution of the large, interfering air peak obtained from the headspace. The bottom chromatogram in Figure 2 shows an overlay of the SPME and headspace chromatograms for acceptable sample A1; the difference is dramatic.

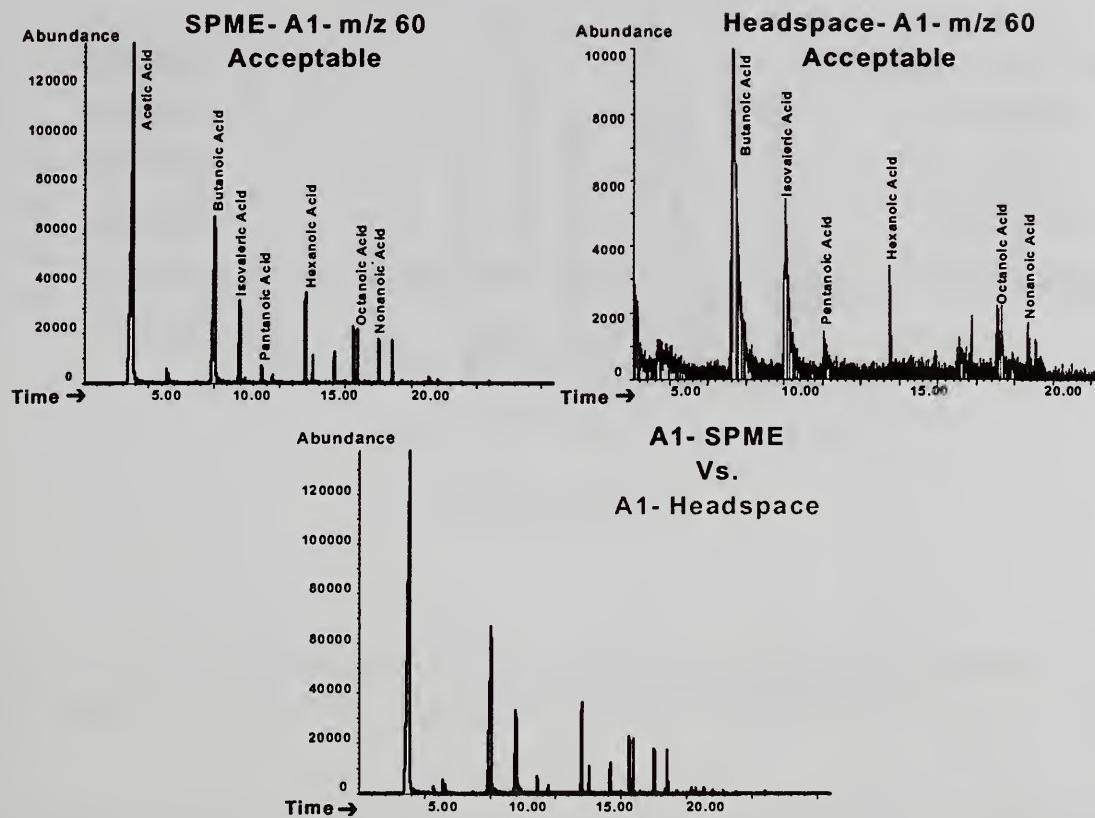


Figure 2. Chromatograms of SPME (upper left) and headspace (upper right) of an acceptable sugar (A2). Bottom - overlay of SPME and headspace chromatograms.

A representative sample of the SPME and headspace chromatograms of a reject sugar (E1) is shown in Figure 3. The upper left chromatogram shows the SPME analysis. Compared to acceptable sugar A1 (Figure 2), there are much higher abundance levels of acetic, butanoic, and isovaleric acids, ranging as high as 260,000 counts for acetic acid. Reject sugars appeared to have lower concentrations of the higher volatile acids, such as hexanoic and octanoic, but this was attributed to the fact that the fiber was probably saturated by the high levels of acetic, butanoic and isovaleric acids present. Headspace analysis is shown in the upper right of the figure. Although headspace analysis clearly reflected the higher levels of butanoic and isovaleric acids in this sample, the maximum abundance measurement was only about 22,000. The bottom chromatogram in Figure 3 shows the overlay of SPME and headspace, again demonstrating the large difference in sensitivity for the two methods.

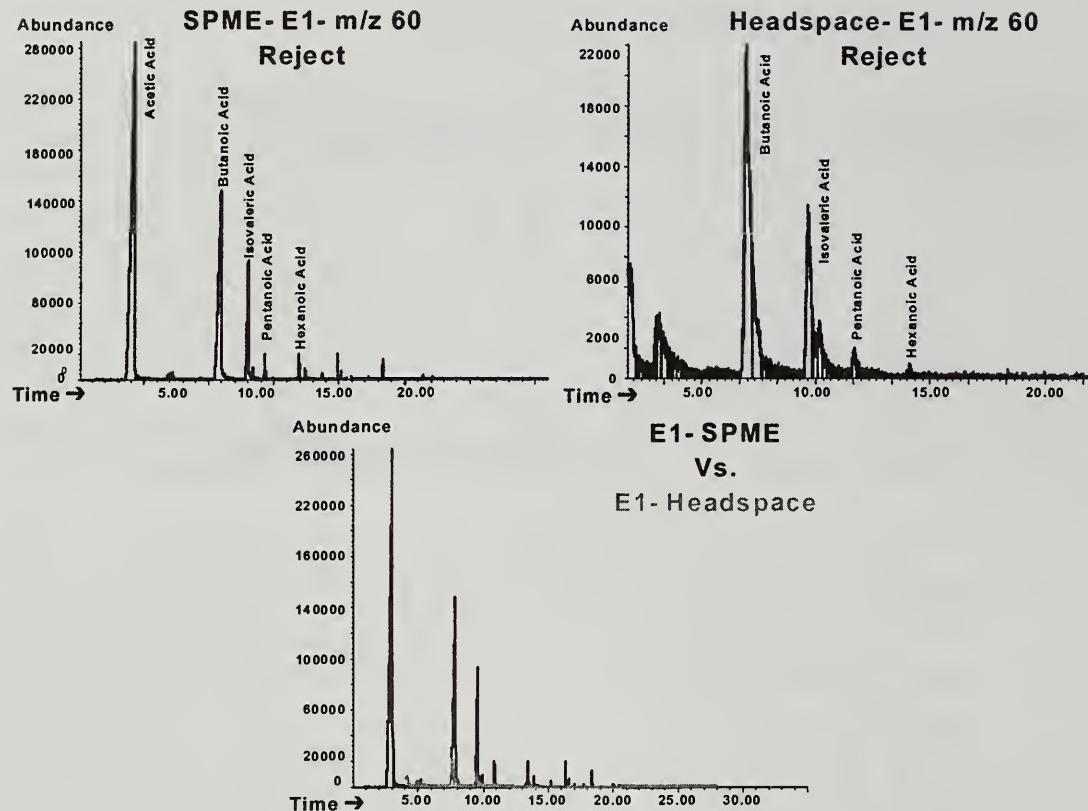


Figure 3. Chromatograms of SPME (upper left) and headspace (upper right) of a reject sugar (E2). Bottom - overlay of SPME and headspace chromatograms.

Figure 4 shows a SPME comparison for two acceptable sugars A1, A2), two borderline sugars (B3, D4), and two reject sugars (E1, E2). The reject sugars have much higher levels of acetic, butanoic, and isovaleric acids. Borderline sugar D4 had a high level of acetic acid, but low levels of butanoic and isovaleric acids. In this case, acetic acid could be suspected of helping to move this sugar into the borderline category.

Figure 5 shows a headspace analysis comparison for the same samples as shown in Figure 4. It is evident that headspace analysis tracks a similar trend as SPME, except that the abundance measurements are only about one-tenth that of the SPME analysis. Headspace clearly shows the higher levels of the most objectionable volatile fatty acids, butanoic and isovaleric acids, in the reject sugars.

Figures 6, 7, and 8 compare SPME and headspace analysis of butanoic acid, isovaleric acid and hexanoic acid in the sugars referred to in Figures 4 and 5. In all cases, not only does SPME show much higher area counts for the VFA than does headspace, but SPME also differentiates the sugars much better than headspace does. Butanoic and isovaleric acid are shown to be much higher in the rejected sugars compared to the acceptable and borderline sugars. Hexanoic acid, by contrast, is higher in the acceptable sugars, although the hexanoic acid maximum abundance for all the sugars (10,000 - 40,000) is quite a lot lower than butanoic and isovaleric in most of the sugars.

SPME Comparison

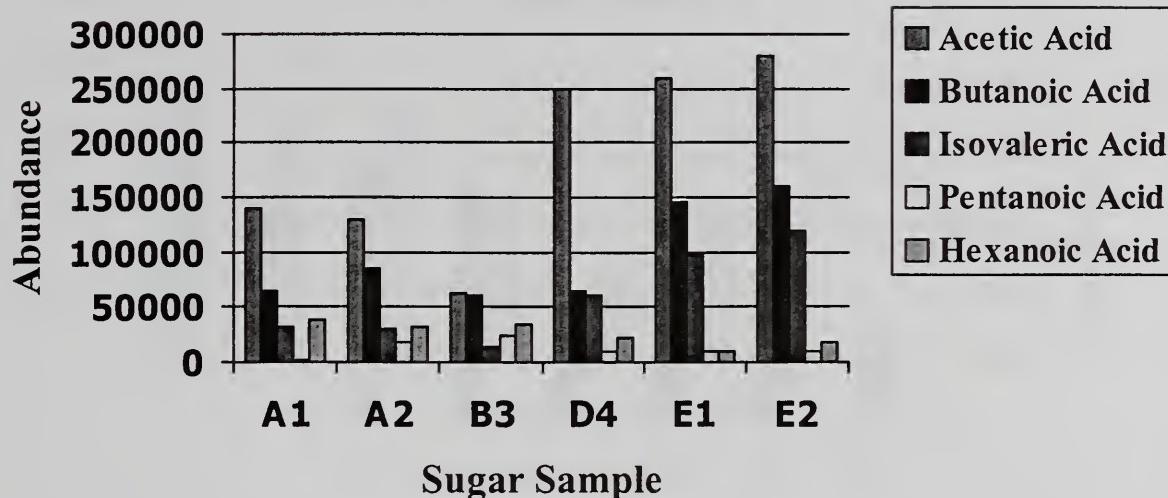


Figure 4. Comparative abundance of VFA by SPME in five sugars. A1 and A2 = acceptable; B3 and D4 = borderline; E1 and E2 = reject.

Headspace Comparison

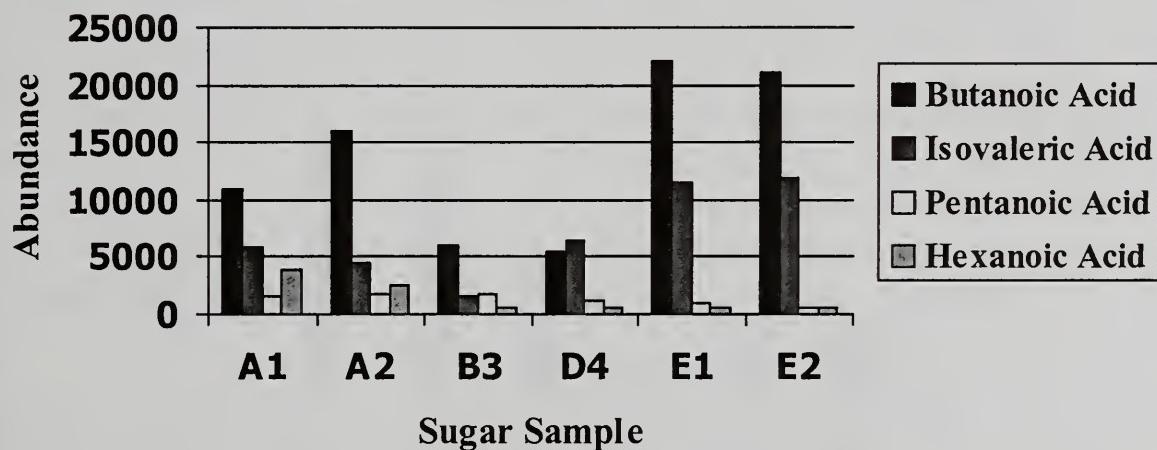


Figure 5. Comparative abundance of VFA by headspace in five sugars. A1 and A2 = acceptable; B3 and D4 = borderline; E1 and E2 = reject.

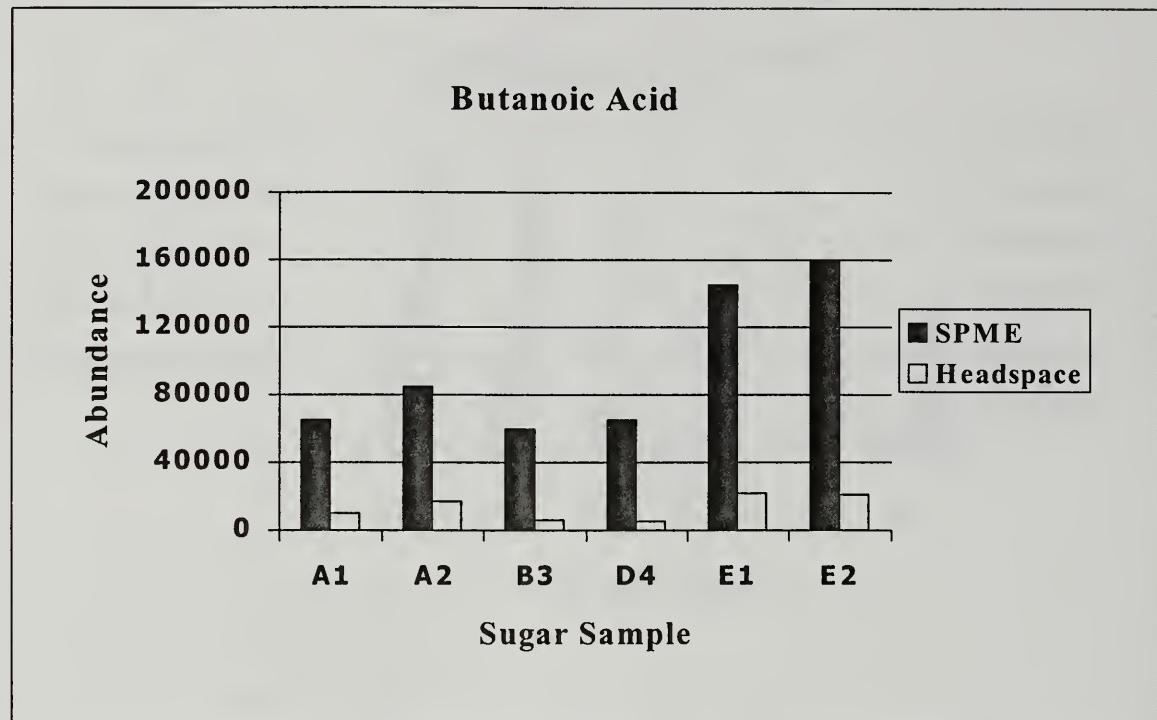


Figure 6. Comparison of butanoic peak area counts by SPME and by headspace in five sugars. A1 and A2 = acceptable; B3 and D4 = borderline; E1 and E2 = reject.

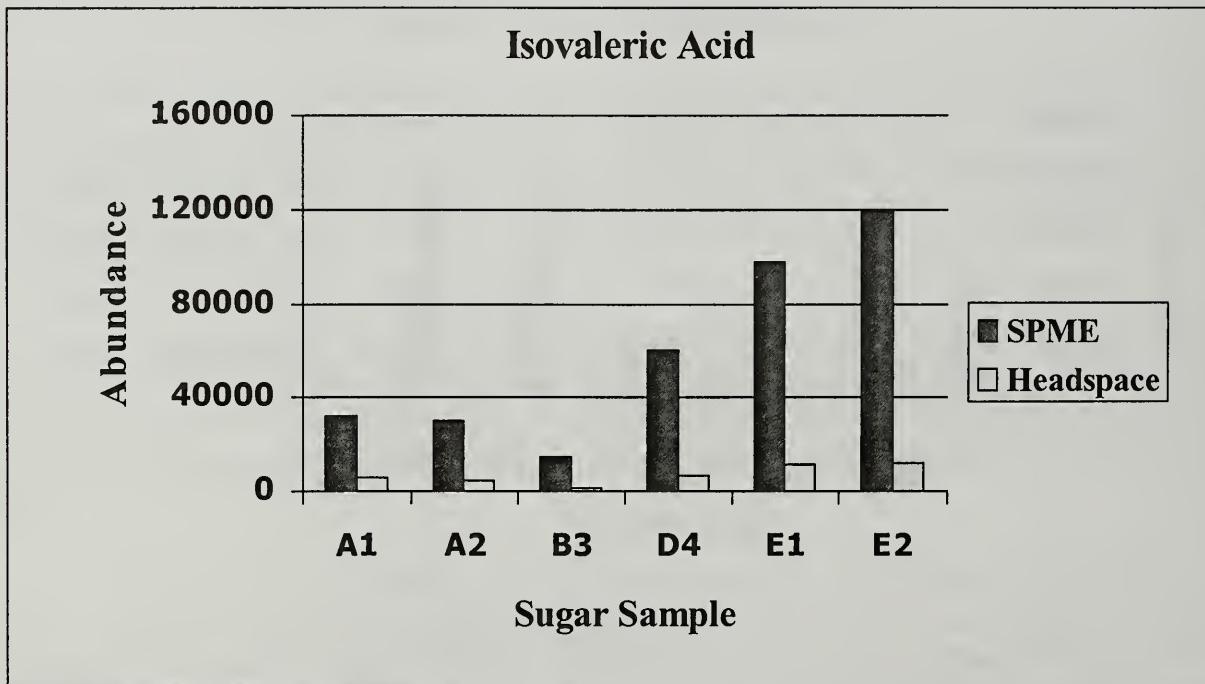


Figure 7. Comparison of butanoic peak area counts by SPME and by headspace in five sugars. A1 and A2 = acceptable; B3 and D4 = borderline; E1 and E2 = reject.

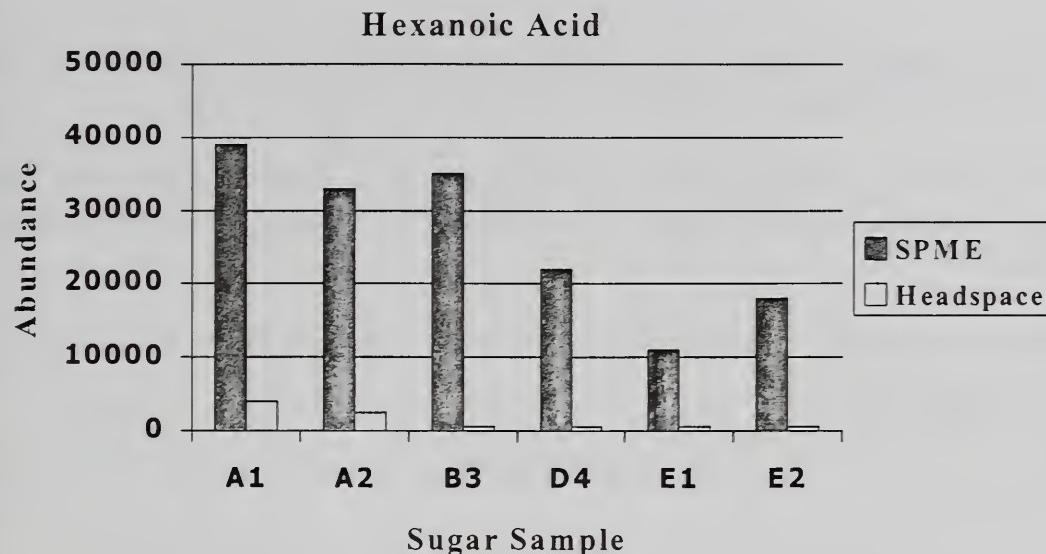


Figure 8. Comparison of butanoic peak area counts by SPME and by headspace in five sugars. A1 and A2 = acceptable; B3 and D4 = borderline; E1 and E2 = reject.

CONCLUSIONS

In conclusion, reject sugars had higher levels of the objectionable volatile fatty acids, butanoic and isovaleric, than did the acceptable or borderline sugars. This was shown to be true for both SPME and headspace. Acetic acid was also much higher in reject sugars, and may serve as a marker for assessing sugars. While headspace analysis showed similar trends to SPME, it was not able to analyze acetic acid because of interference by the air peak, and it was only about one-tenth as sensitive as SPME. Acetic acid is not necessarily always a cause off-odor, but its presence at a high concentration is a good indicator or marker that the other acids will also be present. If, however, there is a sufficiently high concentration of acetic acid, it can produce a strong, sour odor in the sugar. Butanoic and isovaleric acids are responsible for much of the off-odors in white beet sugar, and a rapid method to assess them would be useful to confirm a reject sugar. While quantitation of these acids is desirable, it would not be necessary in a quality control laboratory, as relative abundance could serve as a good indicator. Both SPME and headspace analysis are rapid, environmentally friendly methods. The SPME apparatus is very inexpensive, and given its high level of sensitivity for VFA in beet sugars, would be recommended for quality assessment of beet sugars instead of headspace analysis.

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THE EFFECT OF OZONE AND AIR ON OFF-ODORS IN BEET SUGAR

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ABSTRACT

This study investigates ozone and air as potential polishing agents for the elimination of off-odors periodically found in beet sugars.

Ozone and air were directly circulated through a bed of crystalline sugar to oxidize and/or remove volatile compounds responsible for off-odors. Various experimental parameters, such as treatment time, temperature, pressure and ozone concentration were tested to determine the optimum conditions for ozone or air action. Volatile off-odor compounds were analyzed using solid-phase-micro-extraction/gas-chromatography/mass-spectrometry (SPME-GC-MS). The effect of ozone and air on the volatile fatty acids (VFA) profile was closely monitored, since it has been established by SPRI and other researchers that VFA, when present in beet sugar, are a significant source of off-odors and off-flavors, even at very low levels.

Results showed that air was more efficient than ozone in removing odoriferous compound from crystalline beet sugar. At optimal conditions, air removed all compounds to a very large extent, whereas ozone did not and ultimately induced formation of acetic acid. Air treatment is also a more economical solution.

INTRODUCTION

Good sensory quality is of major economical importance in the sugar industry and in the food industry in general. Off-odors and off-flavors, even at low levels, greatly affect the perceived quality of sugar, diminishing its potential utilization by the food industry, leading to a decrease in its selling value or even to rejection by the customer.

Off-odors exuding from white beet sugars can be caused by a wide array of over 50 different volatile compounds, such as pyrazines, aldehydes, alcohols, furans, phenolics and carboxylic acids (1). Short chain volatile fatty acids (VFA), in particular, acetic, propanoic, isovaleric and butanoic acids were found to be off-odor compounds of major importance for they can cause odor and flavor at very low concentrations in crystalline beet sugar (1,2). Malodorous chemicals in beet sugars originate from the activity of soil microorganisms or from the beet itself (indigenous or absorbed) or are formed during the manufacturing process (caramelization, Maillard reaction) (1,2,6).

The bulk of the odoriferous organic compounds is located at the surface of the crystals, trapped in a thin film of concentrated syrup surrounding the crystals. Sugar crystals grow in supersaturated syrup, which is removed by centrifugation once the proper crystal size is attained. Most of the syrup is separated from the crystals using water or steam spraying, but a very thin layer of syrup always remains around the crystals. This surface layer of saturated syrup contains most of the impurities and water present in the whole crystal, including the volatile odoriferous compounds (6).

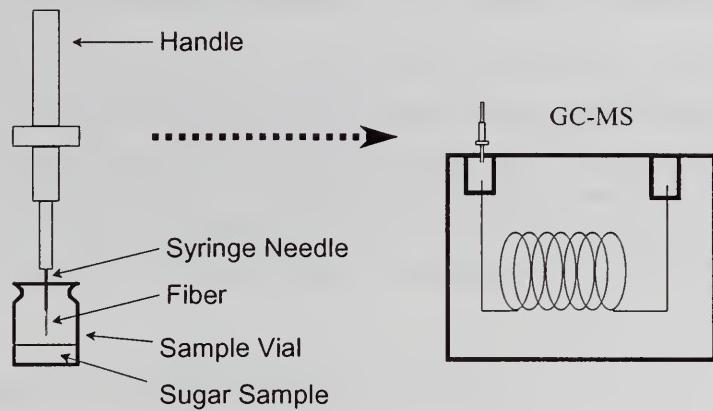
Research on off-odors in white sugar concerns essentially the analytical dimension of the problem, and great efforts have been made to develop a rapid technique to extract and analyze trace volatile compounds from crystalline white sugar. GC-MS is the ideal method to identify the off-odor volatile compounds, and, in spite of some limitations, headspace analysis (3) is the best way to extract the volatiles from the sugar. SPME (Solid Phase Micro Extraction) offers rapidity, economy, and concentration benefits over simple headspace (4).

SPME utilizes a fiber coated with an adsorbent material that is placed in the headspace of a sample for a specific period of time and temperature. During this time, the volatiles in the headspace are concentrated on the thin film of the fiber. The fiber is subsequently desorbed with heat into a gas chromatograph-mass spectrometer (GC-MS) for sample analysis and identification of the volatiles (Figure 1).

A recent S.P.R.I. comparative study showed that SPME is a more sensitive extraction technique for volatiles in crystalline sugars than headspace (3). SPME had especially high sensitivity for VFA, which are some of the main odoriferous agents (3). SPME GC-MS is an environmentally friendly procedure that requires a small amount of sample, uses no solvents, and is accurate, efficient, economical, simple, quick and allows semi-quantitative analysis (5).

Solid Phase Micro Extraction

Figure 1. Solid Phase Microextraction principle.



Very little literature has been published concerning the process side on how to eliminate or attenuate off-odors in white beet sugars. It was reported that partial elimination of malodor was achieved by ventilation of the beet sugar during bulk storage (6) as well as by air sweep in silos. Various other treatment suggestions included porous absorbent, additional washing in the centrifuge and treatment with polishing carbon (7). In recent years SPRI had begun to investigate potential applications of ozone in the sugar industry, looking to the time when ozone might be economically viable due to modern ozone generation plants being able to produce ozone cheaply, reliably and in high concentrations, favoring good reaction kinetics (8). Ozone received classification as a GRAS (Generally Recognized as Safe) substance by the U.S. Food and Drug Administration in 1982, allowing its broad use in the food industry.

It was decided to test ozone as a polishing agent for removal of off-odors in beet sugar. The principle of air sweep or air circulation in silos was used in the experimentation as it seemed to be the most practical way to apply ozone.

The primary objectives of this study were to determine and optimize the potential action of ozone treatment for off-odor removal in beet sugars, using SPME GC-MS as a qualitative measurement system. A parallel study was conducted with air treatment.

EXPERIMENTAL

Ozone was supplied by an electrical generator (120-V, 60-Hz, 0.45-A), which produced 0.45 g/hr of ozone, using an ambient air supply of 20-SCFH (Standard Cubic Feet per Hour). During treatment, the sugar samples were placed in a vertical double-jacketed glass column connected to a water bath to heat the sugar. The ozone and air streams circulated through the sugar bed from the bottom to the top of the column. 50 g of sugar was treated in each experiment. After treatment the sugars were spread on a glass dish for cooling and stored in glass containers.

Five sets of 4 to 7 beet sugars from five different North American locations (A, B, C, D, E) were used in the experiments. Each sugar was sensory scored by three individuals, looking at a set of descriptors. Each sugar was given an over-all sensory rating, based on resolving the sensory data into three categories: Acc = acceptable; Bor = borderline; Rej = reject.

SPME GC-MS was performed on 5 g samples placed in 10 ml air sealed vials. The vial was agitated for 15 minutes at 65°C at 100 rpm (10 seconds of agitation separated by 1 second pauses). The fiber was exposed to the headspace for 15 minutes, with the same agitation and temperature. GC and MS conditions have been described earlier (3). The fiber was desorbed for 2 minutes in the injection port of the gas chromatograph at 270°C. The fiber was regenerated by baking it in a fiber heater in the presence of a helium purge at 270°C for 1 minute.

RESULTS AND DISCUSSION

All untreated sugars used in this study had similar chromatographic patterns, with two major groups of volatile products detected (Figure 2). The first group included short-chain volatile fatty acids of rather low abundance (concentration), with retention times of 1 to 7 min. The second group, with longer retention times, was made up of compounds with higher molecular weight and greater abundance, with retention times of 22 to 30 min. There were only a few intermediate compounds of significant abundance in between those two groups. (Peaks marked as contaminant arise from the SPME fiber or the column.)

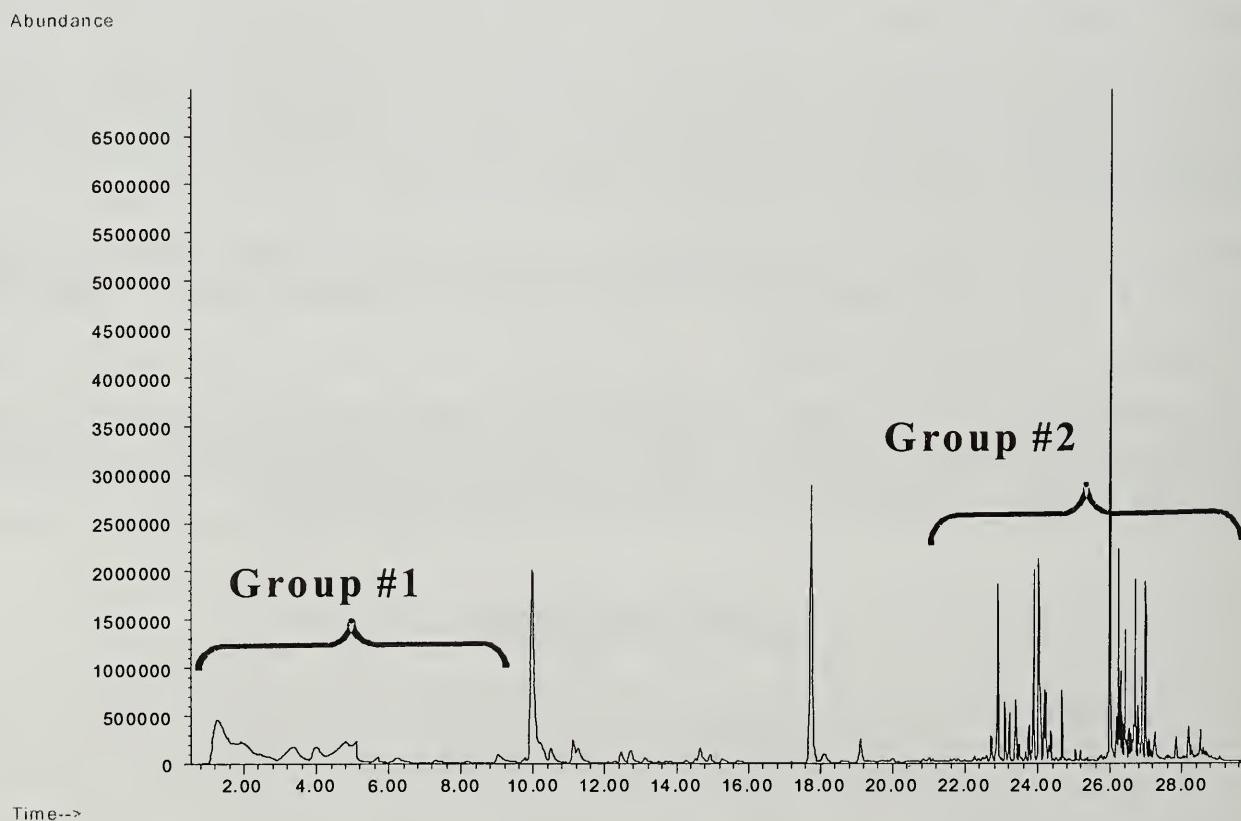


Figure 2. Sugar D6_{Acc}, no treatment at room temperature (RT), total ion chromatogram (TIC).

For better definition, the mass fragment of molecular weight 60 (*m/z* 60) was used to scan specifically for VFA and other fatty acids. With the exception of propanoic acid, the base peak of short-chain fatty acids is *m/z* 60 (4). Analysis at ion *m/z* 60 showed that all the sugars had four VFA in common: Acetic acid, butanoic acid, 3-methyl butanoic acid (isovaleric acid) and pentanoic acid (valeric acid), as shown in Figure 3.

These fatty acids have strong, characteristic odors (9) and are responsible for some of the major off-odors in beet sugar (1). Several sugars ($E4_{Bor}$, $C4_{Bor}$ and $C5_{Bor}$) also presented hexanoic acid (9.5 min) and traces of heptanoic acid, octanoic acid and nonanoic acid ($E4_{Bor}$ and $C4_{Bor}$). Hexanoic, heptanoic, octanoic and nonanoic are also odorous agents but less powerful than the VFA (9).

The second group of volatiles was composed mainly of alkanes and compounds of various nature and abundance (Figure 4). They have not been fully identified at the present time.

Effect of Ozone Treatment at Room Temperature.

The ozone generator was supplied with air by a pump with a flow-rate of 0.8 liters/min or 3.3 SCFH, thus producing 0.074 g ozone/hr. In this experiment, three ozone concentrations were tested at room temperature (20-25°C): 240 (10 min), 480 (20 min) and 960 (40 min) ppm (w/w). Only the results at 480 and 960 ppm are discussed because no significant changes were noticed at 240 ppm. Six and beet sugars were treated with 480 ppm ozone: $E3_{Bor}$, $C7_{Bor}$, $E4_{Bor}$, $C5_{Bor}$, $C4_{Bor}$, $D6_{Acc}$; and five were treated with 960 ppm ozone: $D3_{Bor}$, $C1_{Acc}$, $B2_{Bor}$, $A5_{Rej}$, $E1_{Rej}$.

Treatment with ozone showed definite action on the VFA (Figure 3). Tables 1 and 2 show the difference between each VFA peak area before and after treatment for each sugar. The general trend was that acetic, butanoic, 3-methyl butanoic and pentanoic acids were partially removed from the sugar, and hexanoic, heptanoic, octanoic and nonanoic acids were increased or formed. Ozone action increased as ozone dosage was increased.

Table 1. Ozone 480 ppm @ RT: VFA removal/formation (% peak area).

	$E4_{Bor}$	$C4_{Bor}$	$C5_{Bor}$	$E3_{Bor}$	$D6_{Acc}$	$C7_{Bor}$
Acetic ac	12.6 %	14.5 %	12.8 %	50.7 %	51.8 %	42.0 %
Butanoic ac	86.4 %	93.1 %	92.6 %	66.2 %	94.4 %	85.8 %
3-Me Butanoic ac	30.6 %	63.1 %	28.4 %	42.5 %	71.4 %	76.5 %
Pentanoic ac	29.8 %	74.5 %	54.3 %	22.0 %	36.8 %	82.3 %
Hexanoic ac	26.8 %	12.7 %	71.1 %	Formed	79.4 %	Formed
Heptanoic ac	61.0 %	44.5 %	Formed		Formed	Formed
Octanoic ac	71.5 %	87.3 %	Formed		Formed	Formed
Nonanoic ac	89.5 %	Formed	Formed		Formed	Formed

Increased

Decreased

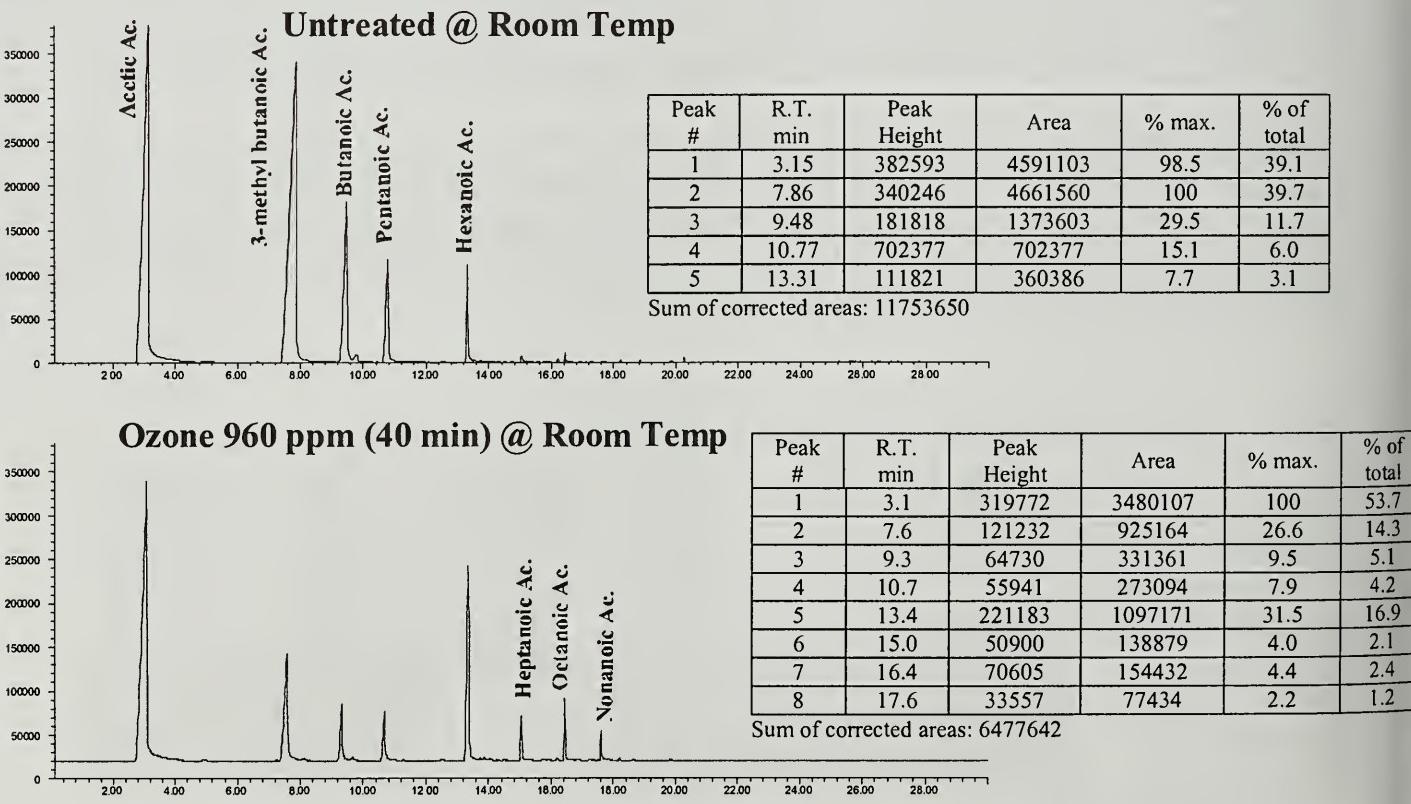
Table 2. Ozone 960 ppm @ RT: VFA removal/formation (% peak area).

	A5_{Rej}	B2_{Bor}	C1_{Acc}	D3_{Bor}	E1_{Rej}
Acetic ac	55.8 %	24.2 %	11.6 %	24.2 %	44.8 %
Butanoic ac	50.8 %	80.2 %	89.1 %	86.0 %	49.5 %
3-Me Butanoic ac	20.7 %	75.9 %	91.1 %	88.2 %	20.7 %
Pentanoic ac	85.0 %	61.1 %	89.1 %	64.8 %	35.6 %
Hexanoic ac	88.9 %	67.2 %	61.8 %	44.1 %	93.5 %
Heptanoic ac	16.4 %	Formed	33.8 %	39.9 %	Formed
Octanoic ac	Formed	Formed	6.5 %	14.2 %	Formed
Nonanoic ac		Formed			

Increased

Decreased

It appeared that ozone removed the highly odoriferous VFA (acetic, 3-methyl butanoic and butanoic acids) and had formed less odoriferous acids (hexanoic, heptanoic, octanoic and nonanoic acids) through oxidation reactions. However, this was not true for all sugar. For example, acetic acid increased in E4_{Bor}, E3_{Bor}, A5_{Rej} and E1_{Rej} and in quite high proportion for the last 3 sugars cited (>45%). Additionally, hexanoic and heptanoic acids were formed in C1_{Acc} as well as heptanoic and octanoic acids in D3_{Bor}.

Figure 3. Sugar B2_{Bor}, ozone treated, 960 ppm at room temperature m/z 60.

Ozone had a marked effect on the compounds of the second group (Figure 4). The primary observation was that, with the exception of butyl-butanoate, which increased in a few cases, all of the HMW compounds were well removed and some were completely eliminated (Table 3-4).

On the other hand, several new compounds of intermediate molecular weight appeared on the chromatographs with low to moderate abundance. These compounds were very likely ozone oxidation products created from the HMW compounds, especially the alkanes. The most significant of the newly formed compounds were nonanal and decanal, which are listed in Tables 3 and 4. These were common to all the sugars tested. Dodecanal consistently increased with ozone treatment or was significantly formed. Dodecanal was detected at very low level in some untreated sugars and was absent in the others (Tables 3 and 4).

Unlike butyl-butanoate, the three aldehydes (nonanal, decanal, and dodecanal) are powerful odoriferous agents (9), especially nonanal and decanal, and are likely to cause off-odors in the treated sugar. Hence, although HMW compounds were substantially decreased or even eliminated, the ozone action was not satisfactory under these conditions since mild or strong odoriferous agents were newly formed probably due to incomplete oxidation of other compounds already present. Despite the presence of new compounds, sensory analysis of treated sugar did not detect off-odors, probably because they were present in subthreshold concentrations.

Abundance

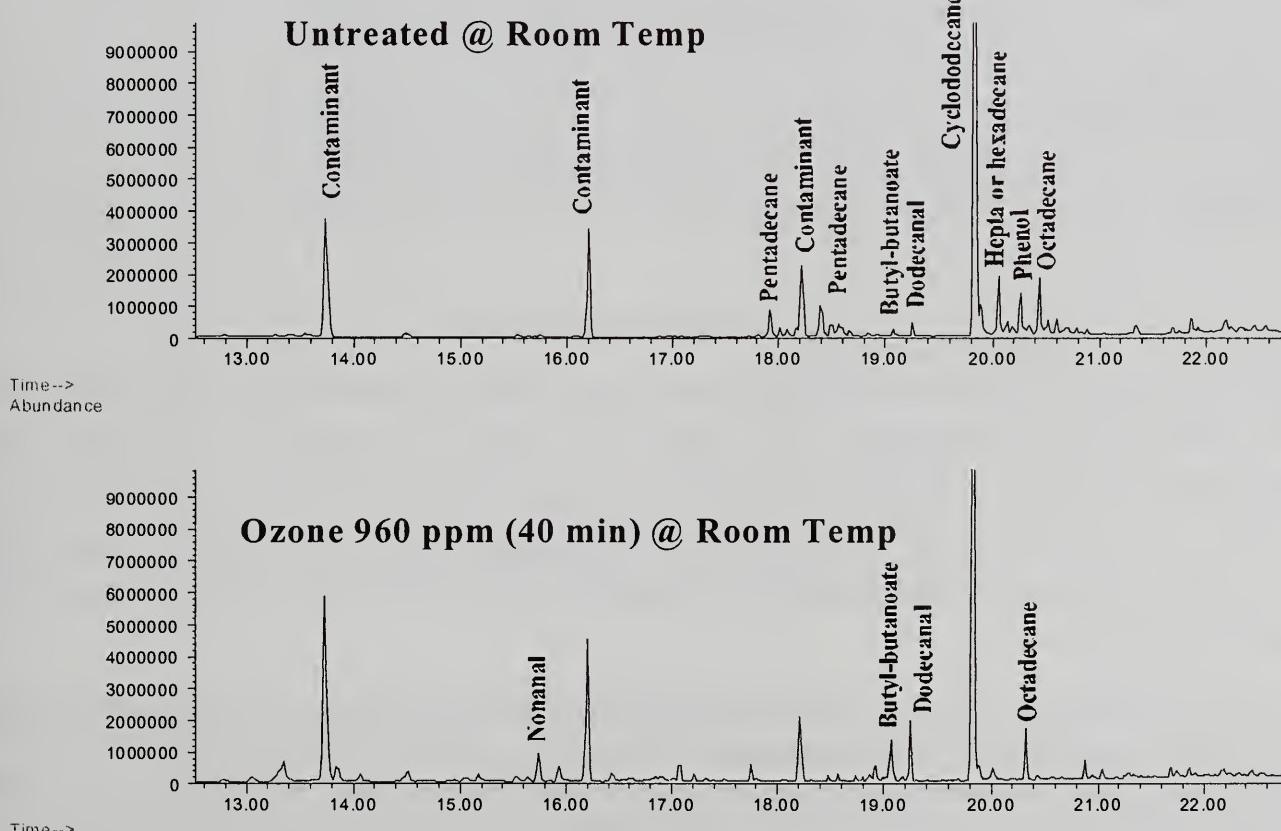


Figure 4. Sugar A5_{Rej}, ozone treated, 960 ppm at room temperature, TIC.

Table 3. Ozone 480 ppm @ RT: HMW removal/formation (based on peak area).

	E4_{Bor}	C4_{Bor}	C5_{Bor}	E3_{Bor}	D6_{Acc}	C7_{Bor}
Nonanal	Formed	Formed	Formed	Formed	Formed	Formed
Decanal	Formed	Formed	Formed	Formed	Formed	Formed
Pentadecane	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
Butyl butanoate	32.6%	3.6%	37.5%	68.3%	52.8%	69.7%
Dodecanal	Formed	Formed	Formed	Formed	Formed	Formed
Cyclododecane	68.6%	16.4%	3.2%	59.5%	12.8%	52.2%
Hepta/Hexadecane	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
Phenol	100%	100%	100%	100%	100.0%	100%
Octadecane	100%	46.4%	64.6%	39.4%	65.2%	58.4%

Increased

Decreased

Table 4. Ozone 960 ppm @ RT: HMW removal/formation (% peak area).

	A5_{Rej}	B2_{Bor}	C1_{Acc}	D3_{Bor}	E1_{Rej}
Nonanal	Formed	Formed	Formed	Formed	Formed
Dodecanal					Formed
Pentadecane	100.0 %	100.0 %	100.0 %	100.0 %	100.0 %
Butyl butanoate	83.7 %	34.6 %	61.8 %	48.9 %	Formed
Dodecanal	76.4 %	57.6 %	76.0 %	62.1 %	56.1 %
Cyclododecane	24.8 %	57.8 %	65.3 %	61.9 %	56.7 %
Hepta/Hexadecane	100.0 %	100.0 %	100.0 %	100.0 %	100.0 %
Phenol	100.0 %	100.0 %	100.0 %	100.0 %	100.0 %
Octadecane	25.0 %	49.3 %	74.5 %	49.7 %	35.7 %

Increased

Decreased

Effect of Heat on Ozone Treatment.

In this experiment, the effect of the temperature of sugar during ozone treatment was tested. The goal in increasing the temperature of the sugar was to reduce the viscosity of the syrup layer surrounding the crystals to facilitate the mobility of the off-odor compounds to make them more accessible to the action of ozone as well as to increase the sweeping action. Sugars D3_{Bor}, C1_{Acc}, B2_{Bor}, A5_{Rej}, E1_{Rej} were used, with the same experimental conditions as above, except that, before ozone treatment, the temperature of the sugar was raised to 75-80°C by circulation of hot water in the column jacket.

Increasing the temperature greatly improved the removal of VFA (Figure 5). As shown in Figure 6, all HMW compounds were also eliminated. After treatment with 960 ppm ozone at 75-80°C, all VFA were largely or even totally removed except acetic acid which concentration was at least doubled in each sugar (Table 5). This indicates that acetic acid may be the final oxidation product of the HMW compounds by ozone. Sensory analysis showed that all the treated sugars were odor-free after treatment, indicating that the acetic acid was below the sensory detection threshold.

Table 5. Ozone 960 ppm @ 80°C: VFA removal/formation (% peak area).

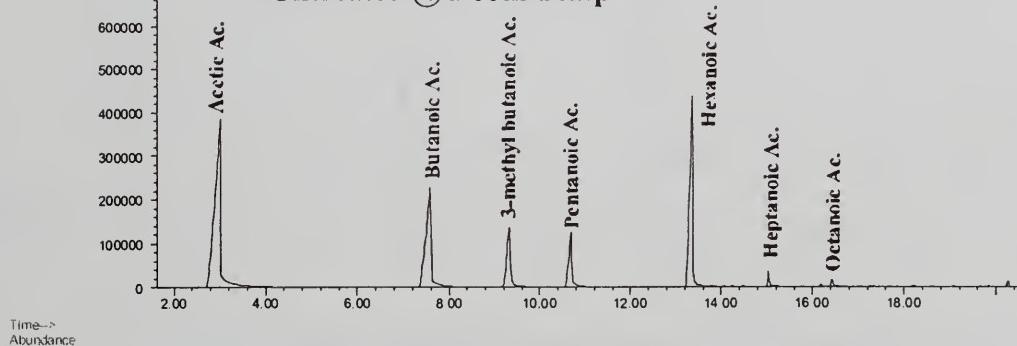
	A5_{Rej}	B2_{Bor}	C1_{Acc}	D3_{Bor}	E1_{Rej}
Acetic ac	69.5 %	50.2 %	54.1 %	59.3 %	51.5 %
Butanoic ac	10.1 %	48.1 %	35.4 %	7.4 %	36.5 %
3-Met Butanoic Ac	54.4 %	72.1 %	82.3 %	64.5 %	65.0 %
Pentanoic ac	19.4 %	51.6 %	73.9 %	43.5 %	48.3 %
Hexanoic ac	22.3 %	58.1 %	84.0 %	62.6 %	100.0 %
Heptanoic ac	100 %	100 %	100 %	100 %	100 %
Octanoic ac	100 %	100 %	100 %	100 %	100 %
Nonanoic ac	100 %	100 %	100 %	100 %	100 %

Increased

Decreased

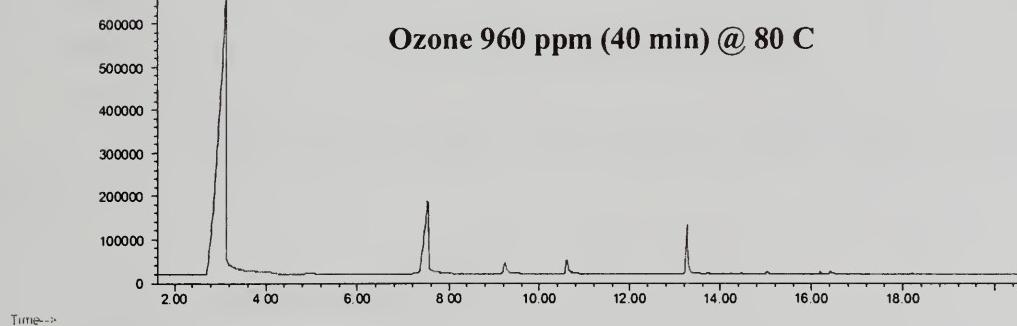
Abundance

Untreated @ Room Temp



Time-->

Ozone 960 ppm (40 min) @ 80 C

Figure 5. Sugar C1_{Acc}, ozone treated, 960 ppm at 80°C, m/z 60.

Abundance

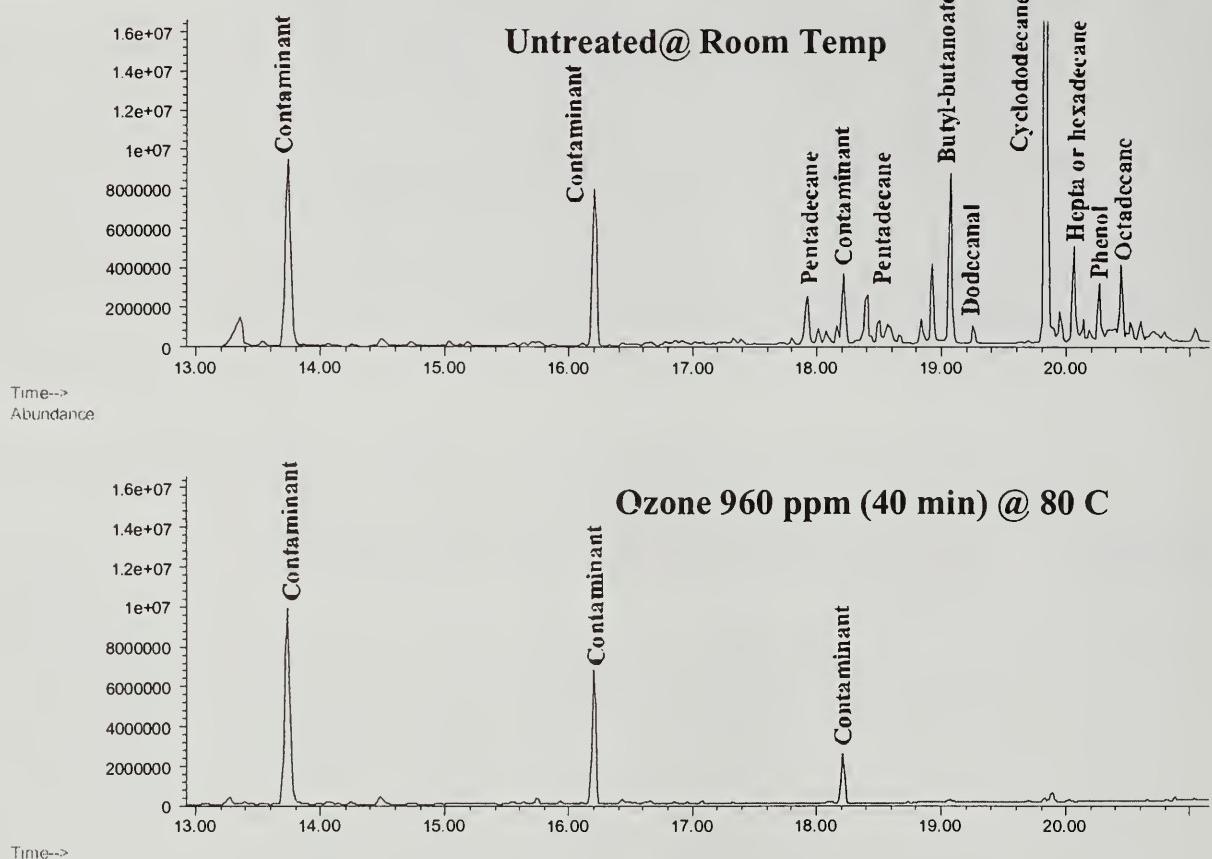


Figure 6. Sugar C1_{Acc}, ozone treated, 960 ppm at 80°C, TIC.

Air Treatment.

Considering that air is the major portion of the ozone stream and since air ventilation is already used in some factories to remove off-odors, the deodorizing effect of air sparging alone was examined. One sugar, E1 containing a wide variety of volatile compounds and rated "borderline to reject" in sensory quality, was treated with air for 40 min, both at room temperature and at 80°C (Figure 7). There was very little effect at room temperature on the VFA and some decrease of the compounds at the higher retention times. With elevated temperature, there was a significant decrease observed in all the volatiles, to the extent that most of them were eliminated, especially VFA (Figure 8) with even acetic acid being largely removed. Unlike ozone at 480 and 960 ppm, no new compounds were formed.

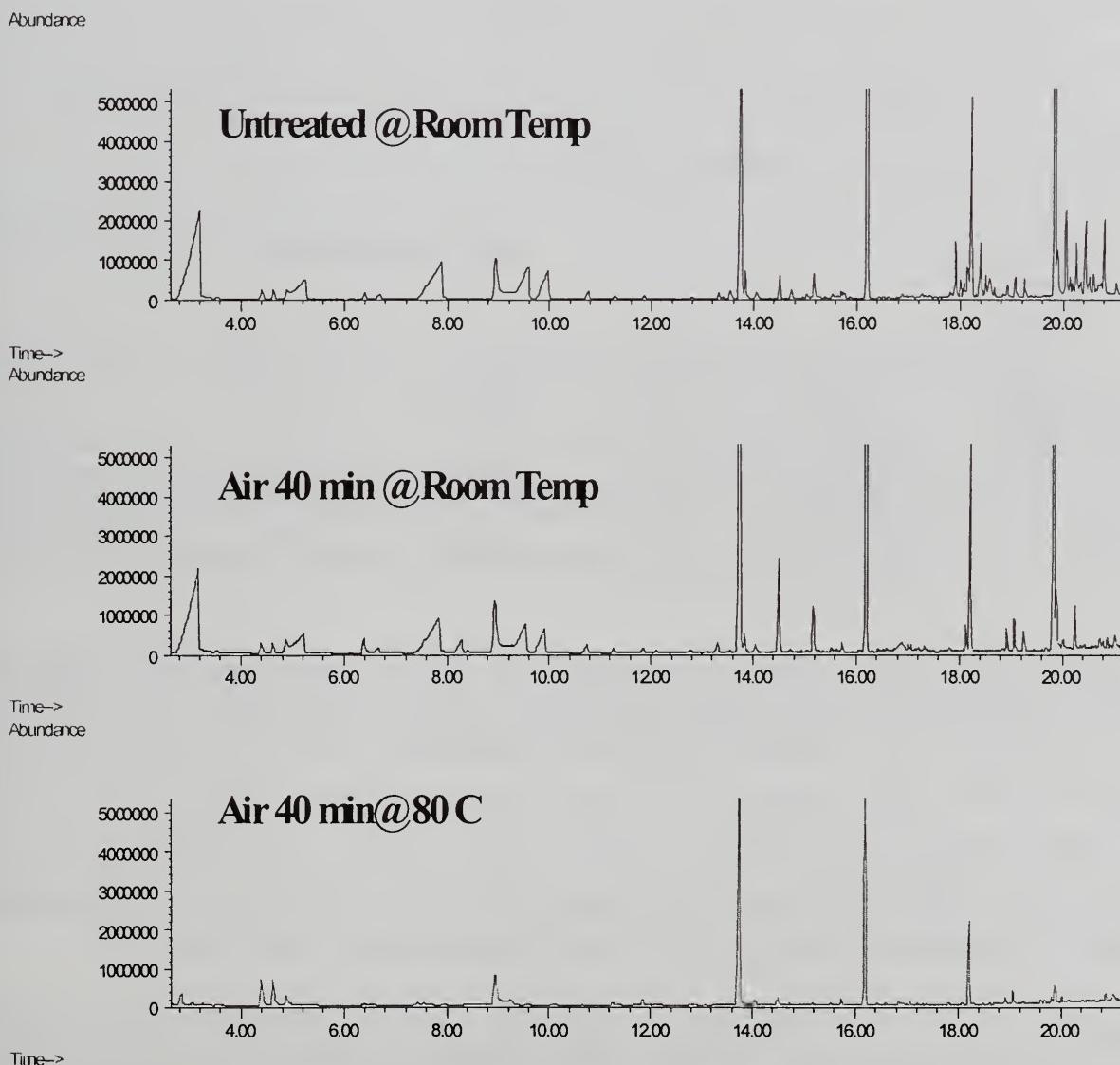


Figure 7. Sugar E1_{Rej}, treated with air at room temperature and 80°C, TIC.

Sensory analysis showed that E1_{Rej} still had a faint residual off-odor after air treatment. However, air treatment produced a sugar that was no longer in the "reject" category, but very close to commercial quality. This may suffice to treat sugars with less odor than E1_{Rej}.

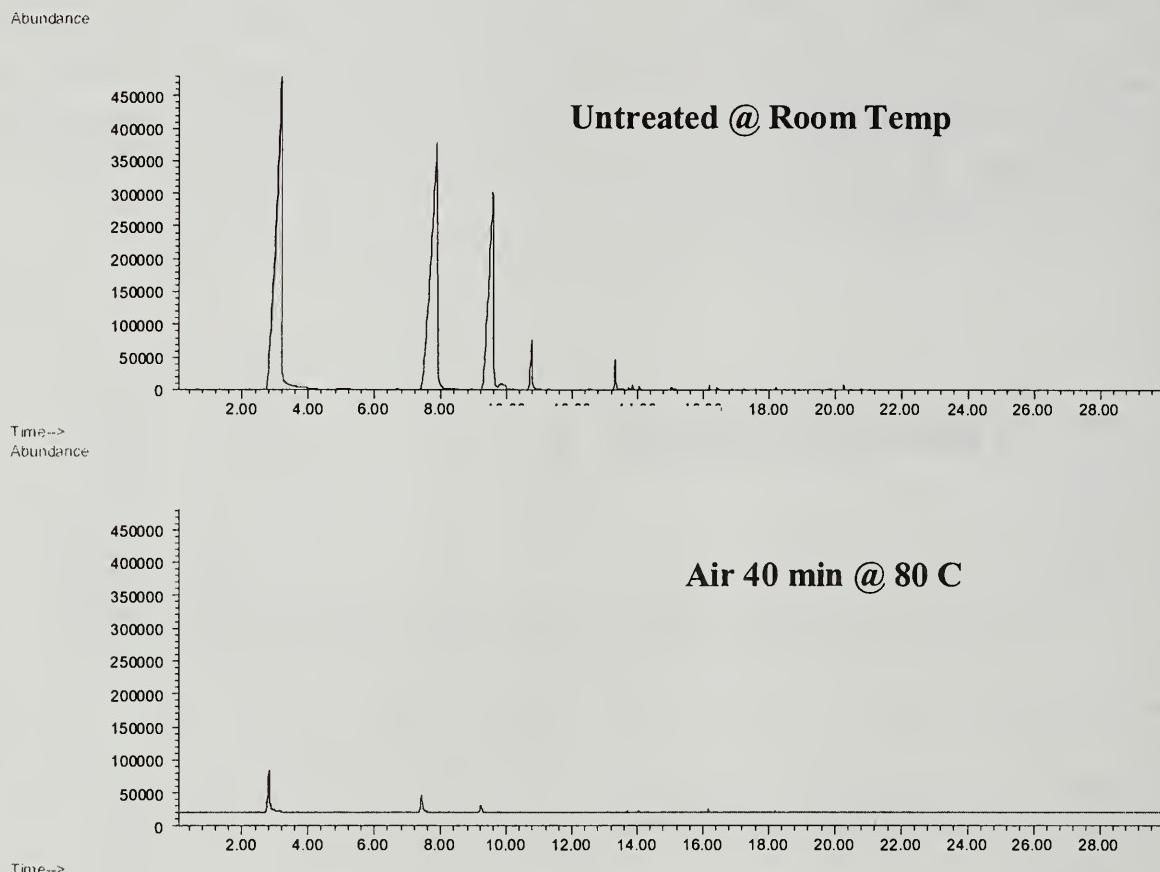


Figure 8. Sugar E1_{Rej}, air treated 40 min at 80°C, m/z 60.

These results indicate that ozone and air treatment at elevated temperature (80°C) may hold promise as deodorizing agents for refined beet sugar. This is a very simple process that could easily be installed in a sugar factory. Ozone or air treatment would be most efficient energy-wise if applied to the sugar coming out of the centrifugal in the factory, which is approximately 65-70°C, hence saving heating energy. However, the relatively high humidity of the sugar coming out of the centrifuges may pose a problem. Hence, ozone and air would most likely be applied batch-wise either at the factory subsequently to the dryer (25-30°C), or by the industrial user (ambient temperature), as needed. In both cases some heating would be required.

The formation of acetic acid with ozone treatment is a major drawback to its use, for it is unlikely that ozone will have any oxidative effect on acetic acid due to its chemical structure ($\text{CH}_3\text{CO}_2\text{H}$). Ozone primarily breaks phenol rings, amines and double bonds in the carbon chain (10), none of which are present in the acetic acid molecule

Effect of Pressure on Air and Ozone Treatments.

Additional testing was conducted in order to confirm the results obtained above for both air and ozone treatments. Malodorous sugars were treated with an excess of ozone for maximum oxidation. Pressurized air was used instead of the pump in order to increase the generator's ozone production. Although the production rate was not known, for a given amount of sugar, by varying either the duration of treatment or the inlet pressure and leaving the other one constant, a relationship between the ozone dosage of several experiments could be established. Air speed was measured through a pressure gauge placed before the sugar bed. Sugar E2, rated "reject" by sensory analysis was treated at 80°C. The amount of sugar (50 g) and the air pressure before the sugar bed (25 in) were kept constant and only the treatment duration time was varied (20 and 40 min). Air experiments were realized with the same experimental conditions.

As previously observed, ozone and air treatments thoroughly removed the HMW at either 20 and 40 min. Also, air largely eliminated VFA (>80 %) at 20 min (Figure 9), and this removal was only slightly increased during 40 min treatment. On the other hand, ozone did not have a positive effect on VFA as can be seen in Table 6. First, ozone increased the amount of acetic acid at 20 min (>40%) and 40 min (>55%). Except for 3-methyl butanoic acid, which was well removed at both dosage rates (>35 %), removal rates for the others VFA were very low at 20 min (<10 %) and these appeared to be formed at 40 min.

Table 6. Ozone 960 ppm @ 80°C: VFA removal/formation (based on peak area).

	Air 20'	Air 40'	O ₃ 20'	O ₃ 40'	
Acetic ac	80.7 %	86.9 %	43.5 %	56.1 %	Increased
Butanoic ac	87.5 %	90.6 %	10.4 %	11.0 %	
3-Met Butanoic ac	91.8 %	91.8 %	41.8 %	34.6 %	
Pentanoic ac	90.6 %	92.5 %	7.3 %	7.3 %	Decreased

These results confirmed that air alone has a more thorough action than ozone since air greatly removed the VFA whereas an excess of ozone did not and even caused their formation. No off-odors were detected by sensory analysis after treatment with ozone.

Abundance

Ion 60.00 (59.70 to 60.30); SPRI 109.D

E2 untreated, RTTime-->
Abundance

Ion 60.00 (59.70 to 60.30); SPRI 110.D (*)

E2, air 20', 25 in, 80 C

Time-->

Figure 9. Sugar E2_{Rej}, air treated 20 min at 25 in and 80°C, m/z 60

Effect of Storage on Treated Sugars.

As mentioned in earlier discussion, despite the formation of acetic acid, sugars treated with ozone had little detectable odors. Acetic acid has a higher sensory threshold than other VFA, which would account for sugars with measurable amounts of acetic acid having little or no odor. Since ozone and air treatment are essentially surface treatments, it is possible that volatiles present deeper in the syrup layer around the crystal or even inside the crystal may eventually diffuse to the surface and again cause the sugar to have an odor. To test this, three beet sugars, A6_{Acc}, B2_{Bor}, B6_{Rej}, were treated with ozone and air for 40 min at 25 in pressure and at 80°C. 50 g of sugar were used in each experiment. After treatment, each sugar was cooled on plastic dishes and then put into clean, oven-dried 250 ml sealed glass containers. Sniff tests were performed right after treatment when sugars had cooled and on days 1, 5, 12 and 20.

As shown in Table 7, off-odors became noticeable in the ozone-treated sugar in less than 20 days. There was little odor re-appearance on the 20th day in air-treated sugars. This is further demonstrated in Table 8, where air showed much higher percentage of removal after 20 days compared to ozone, except for the borderline sugar for which acetic acid removal was a little higher with ozone than with air. This result may indicate that residual ozone in the sugar temporarily masks some of the off-odors.

Table 7. Reappearance of off-odor after air and ozone treatment: Sniff tests.

	A6 (acceptable)		B2 (borderline)		B6 (reject)	
	Air	Ozone	Air	Ozone	Air	Ozone
No treatment	-	++	-	+++	-	++++
Day 0	-	-	-	-	-	-
Day 1	-	+	-	+	-	+
Day 5	-	+	-	+	-	+
Day 12	-	+	-	+	-	+
Day 20	+	++	+	++	+	+++

(-) no off-odor; (+) very subtle off-odor; (++) light but clearly noticeable; (+++) off-odor; (++++) strong off-odor

Table 8.VFA's removal (%) after 20 days, Air vs. Ozone

	Acceptable		Borderline		Reject	
	O ₃	Air	O ₃	Air	O ₃	Air
Acetic ac	38.2	76.1	54.2	46.5	48.9	82.5
Butanoic ac	24.4	82.9	61.6	79.5	37.5	90.0
3-Met Butanoic ac	39.1	83.5	77.0	87.9	65.2	91.0
Pentanoic ac	15.7	85.9	60.3	87.2	46.8	90.0
Hexanoic ac	49.8	64.4	29.6	92.8	7.8	90.9

Increased

Decreased

Effect of Air Velocity.

Considering that air speed through the sugar bed might have an effect on off-odor removal, 40 min tests at 80°C with air were performed on sugar B1_{Rej}, C3_{Bor} and C6_{Bor} at three different air velocities, which were represented by the pressure measured at the column inlet: 5, 15 and 25 in.

Table 9.VFA removal rate (%) for combined ozone/air treatment.

B1 _{Rej}	5 in	15 in	25 in
Acetic ac	89.7	84.2	82.2
Butanoic ac	92.4	88.6	86.8
3-Met Butanoic ac	94.1	92.1	89.8
Pentanoic ac	95.7	94.2	92.7

C3 _{Bor}	5 in	15 in	25 in
Acetic ac	89.6	88.3	70.4
Butanoic ac	94.5	91.0	78.1
3-Met Butanoic ac	96.7	96.9	81.9
Pentanoic ac	98.3	94.4	88.5
Hexanoic ac	99.6	99.3	97.8

C6 _{Bor}	5 in	15 in	25 in
Acetic ac	87.2	82.6	77.3
Butanoic ac	93.7	87.7	79.3
3-Met Butanoic ac	93.8	84.9	78.8
Pentanoic ac	97.0	93.6	97.5
Hexanoic ac	98.6	98.3	95.8

Results showed that the slower the air speed in the sugar bed, the better the removal of VFA (Table 9). At higher speed it is possible air flow is not uniform throughout the sugar bed and that preferential passageways are created, diminishing the contact between air and sugar. The phenomenon is moderate and the removal decreases less than 10 % while the speed triples.

CONCLUSIONS

Air was shown to be superior on every level to ozone in removing/eliminating volatile off-odors from beet sugars. On the first hand, ozone can totally eliminate high molecular weight compounds at high dosage but only partially removed most of the VFA, ultimately producing acetic acid which accumulates in the sugar resulting in potential off-odor, whereas air removed all the HMW compounds as well as all VFA, including acetic acid, by up to 95%. Air treated sugars remained stable over 20 days unlike the ozone treated sugars.

Both incomplete ozone treatment and treatment with an excess of ozone resulted in the formation of oxidation products.

The second major advantage of air over ozone is economical. Ozone utilization requires expensive equipment and demands specific safety regulation.

It was established at a lab scale that the best air action occurs when the sugar to be treated is hot (80-85°C), combined with very low air velocity through the sugar bed. Further tests need to be done to determine the effect of hot air on cold or wet sugar for, on the factory side, it is easier and more economical to heat air than a large amount of sugar.

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DIFFERENTIATING CANE WHITE SUGAR FROM BEET WHITE SUGAR USING ION CHROMATOGRAPHY PROFILES

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ABSTRACT

Methods for international sugar trade are provided by the International Commission for Uniform Methods in Sugar Analysis (ICUMSA). Recently in Europe, there have been reports of illegal trading in Serbia and Montenegro, whereby the origin of white, refined sugar could not be certified. Other countries in Europe and other parts of the world have also most likely suffered from illegal intermixing of cane white sugar (CWS) with beet white sugar (BWS). A method is, therefore, urgently needed that is (a) capable of distinguishing between CWS and BWS, and (b) can measure the percentage of CWS in a CWS/BWS mixture (final goal). Raffinose and theanderose have been advocated as differential markers. However, raffinose is present in both BWS and CWS (although to a much lesser extent in CWS). Pure theanderose is currently not commercially available, and small IC-IPAD (ion chromatography with integrated pulsed amperometric detection) peaks have been found in BWS samples where theanderose eluted in CWS samples. Low raffinose in conjunction with numerous cane marker peaks across IC-IPAD NaOH/NaOAc 45min profiles of 7°Brix blind BWS/CWS samples were successfully used to detect 20% CWS adulteration. Increasing the °Brix levels to 10 allowed detection of 10% CWS adulteration. Chromatography libraries of CWS, BWS and BWS/CWS samples for direct comparisons will aid adulterant detection. Further studies using chemometric techniques are proposed to enhance adulteration detection. At the least, the use of IC-IPAD profiles can be used as a screening method before the further verification and quantitation with more sophisticated techniques, such as isotope determinations, NMR and DSC.

INTRODUCTION

It has been a longstanding tradition of the International Commission for Uniform Methods in Sugar Analysis (ICUMSA) to provide methods for sugar trade. Recently in Europe, there have been reports (Anon, Zuckerindustrie, 2003; Anon, International Sugar Journal, 2004) of illegal trading in Serbia and Montenegro, whereby the origin of white, refined sugar could not be certified. Other countries in Europe and other parts of the world have also most likely suffered from illegal intermixing of cane white sugar (CWS) with beet white sugar (BWS). A method is, therefore, urgently needed that is (a) capable of distinguishing between CWS and BWS, and (b) can measure the percentage of CWS in a CWS/BWS mixture (final goal). Moreover, if methods are readily available to differentiate CWS/BWS mixtures, then illegal traders may reconsider their practices. Presently, however, there is no ICUMSA method for differentiation.

Although BWS contains much higher levels (<800mg/kg) of raffinose than in CWS and other cane products (Mauch, 1998; Morel du Boil, 1997), its very presence in CWS precludes it to be used alone as a differential marker. Morel du Boil (1997) has advocated the use of theanderose, measured by IC-IPAD as a differential marker because it is present in CWS (<350mg/kg) but not in BWS. Mauch (1998) went on to advocate the use of both raffinose and theanderose. Unfortunately, at present no manufacturer offers pure theanderose for sale, and small IC peaks have been found in BWS samples where theanderose eluted in CWS samples (Guenter Pollach, personal communication).

IC-IPAD profiling has been used to check the authenticity of other food products, including honey (Swallow and Low, 1990) and orange juices. Furthermore, Eggleston and Grisham (2003) have observed distinguishing IC peaks in fresh cane juices that were also present in CWS. This study was therefore, undertaken to see if long (45min) IC-IPAD profiles, following a NaOH/NaOAc gradient method could be used to differentiate BWS/CWS mixtures.

EXPERIMENTAL

IC-IPAD Profiles

See Eggleston and Grisham (2003) for full method. A Dionex BioLC instrument and CarboPac column were used. Eluent conditions were: 100mM NaOH isocratic (0.0-1.1min; inject 1.0min), a gradient of 0 to 300mM NaOAc in 100mM NaOH (1.1-40.0min), and return to 100mM NaOH (40.1-45.0min) to re-equilibrate the column. Final sample °Brix's were measured on a refractometer and °Brix's of samples in a run were standardized, by adding de-ionized water, before analyses, and the samples were not filtered.

BWS, CWS, and BWS/CWS Samples

Most samples were obtained from the SPRI sugar library or from Zuckerforschung Tulln Gersellschaft. Individual sugars in the BWS/CWS mixtures were weighed into a plastic test-tube as a percentage, and then shaken vigorously.

RESULTS AND DISCUSSION

Identification of CWS Samples from Different Geographical Sources

We first looked at the IC profiles for CWS samples from all over the world (Figure 1). Most cane peaks on IC profiles were present in CWS samples from all the different geographical sources, and some are known to form during processing (Eggleston et al, 1997). Therefore, at the present time, IC profiles cannot be used to differentiate the geographical source of the CWS.

Comparison of BWS and CWS Samples

We next compared the IC profiles of six BWS samples (European origin) to two CWS samples (Figure 2). As can be seen in Figure 2a, when the chromatograms were overlaid, there were the obvious low raffinose peaks characteristic of CWSs compared to BWSs. A blow-up of the chromatograph between 23.5-42.0 min in Figure 2b indicated there were numerous other peaks that were characteristic of CWS, which were not background noise.

Use of Blind Samples

With clearly different regions of BWS and CWS samples in Figure 2, we were encouraged enough to test the use of IC profiles to differentiate three blind samples (7° Brix) that were made from the SPRI sugar library, with one containing a mix of BWS/CWS. Results are illustrated in Figure 3. Just a simple overlay of the chromatograms from the three blind samples (Figure 3a), showed that there was a low raffinose peak in sample 3, which was the first clue that it contained CWS. Other CWS clues were visible (Figure 3a), but a blow-up of the chromatograms between 21.2-42.0 min, allowed a 100% correct diagnosis of the three blind samples (see Figure 3b).

The success of the identification of the first three blind samples (Figure 3), spurred us on to test five more blind samples also made from the SPRI library and 7° Brix, ensuring that some BWS/CWS mixes were included. Results are illustrated in Figure 4. Again, just a simple overlay of the chromatograms from the five blind samples (Figure 4a), showed that there was a low raffinose peak in sample 3 with another CWS marker at ~ 21.5 min (Figure 4a) indicating it contained CWS. It was not until we blew up the chromatograms (Figure 4b) that we could identify the samples better. Characteristic CWS marker peaks in Figure 4b allowed an 80% correct diagnosis of the samples (see Figure 4b). Only a 90% BWS/10% CWS mix was not be diagnosed correctly, and this was most likely because of slight shifting to higher retention times (Figure 4). This highlights the need for stable retention times, which can be achieved with column heaters, and standardization of chromatogram patterns using an internal standard.

A further study of seven blind samples (7° Brix) sent to Dr. Eggleston's laboratory in the U.S. from Dr. Pollach in Austria are shown in Figure 5. An 86% correct diagnosis of the seven blind samples was achieved, with an 80%BWS/20%CWS mixture diagnosed incorrectly (see Figure 5). We thought an increase in the $^{\circ}$ Brix level from 7 to 10 could improve this. This was proved right when we subsequently analyzed five more blind samples from the SPRI library (with BWS/CWS mixtures included) injecting a 10° Brix sample onto the IC column (results not shown). Diagnosis was 100% correct and 10% CWS adulteration was detected.

CONCLUSIONS

Most cane peaks on IC-IPAD chromatograms are present in CWS samples from around the world. IC cannot be used to differentiate the geographical sources of the CWS. Low raffinose in conjunction with numerous cane marker peaks across IC-IPAD profiles of 7°Brix blind BWS/CWS samples were successfully used to detect 20% CWS adulteration. Increasing the °Brix levels to 10 allowed detection of 10% CWS adulteration. Detection is also improved when the peak retention times are stable. Constant temperature columns and autosamplers will certainly help to stabilize retention times, but interpretation of the chromatograms will also be greatly assisted with the use of an internal standard. Training of the chromatographer will be needed for careful interpretation of the IC profiles. Chromatography libraries of CWS, BWS and BWS/CWS samples need to be built to aid adulterant detection. Further studies using chemometric modeling are proposed to enhance adulteration detection, by detecting adulterant IC markers that cannot be found visually. At the least, the use of IC-IPAD profiles can be used as a screening method that can detect a range of different compounds in one chromatogram. Suspect samples could then be further verified and quantification with more sophisticated techniques, such as stable isotope determinations (Gucek et al, 1998), nuclear magnetic resonance (NMR) and differential scanning calorimetry (DSC) (Cordella et al, 2002).

ACKNOWLEDGEMENTS

Dr. Les Edye of SRI in Australia is thanked for useful discussions.

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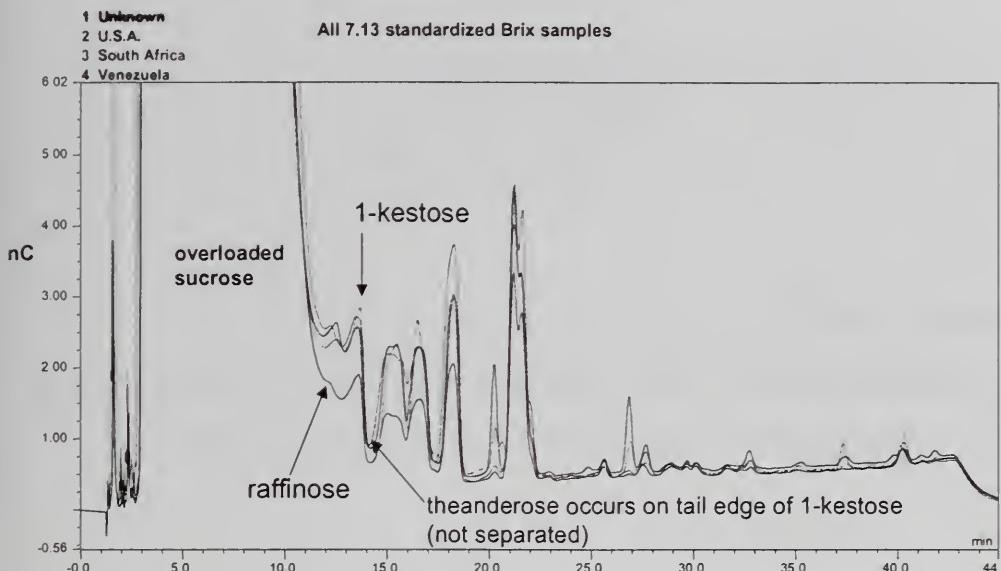


Figure 1. Cane white sugar samples (CWS) from different geographic sources.

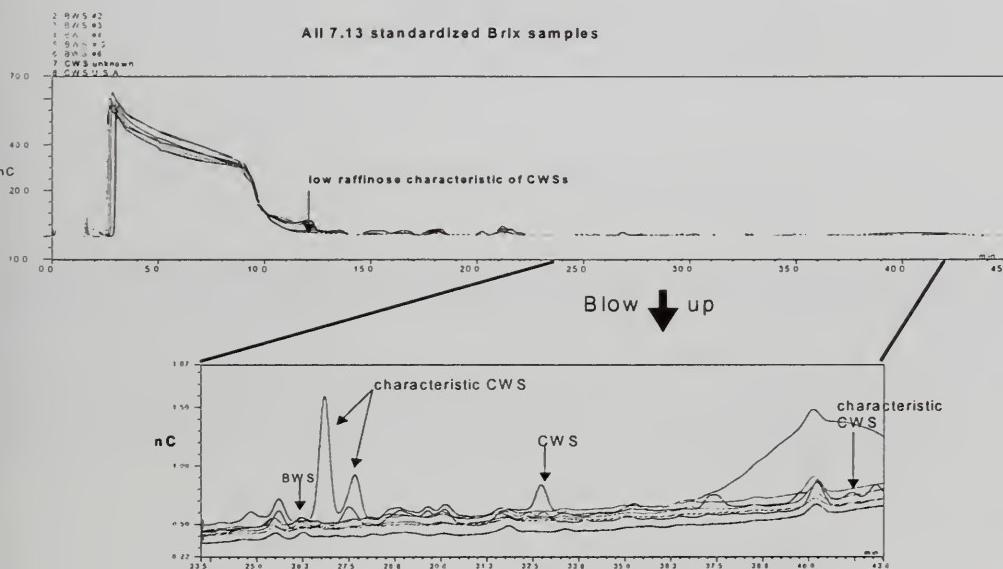


Figure 2. Comparison of six beet white sugar (BWS) samples (European origin) to two CWS samples.

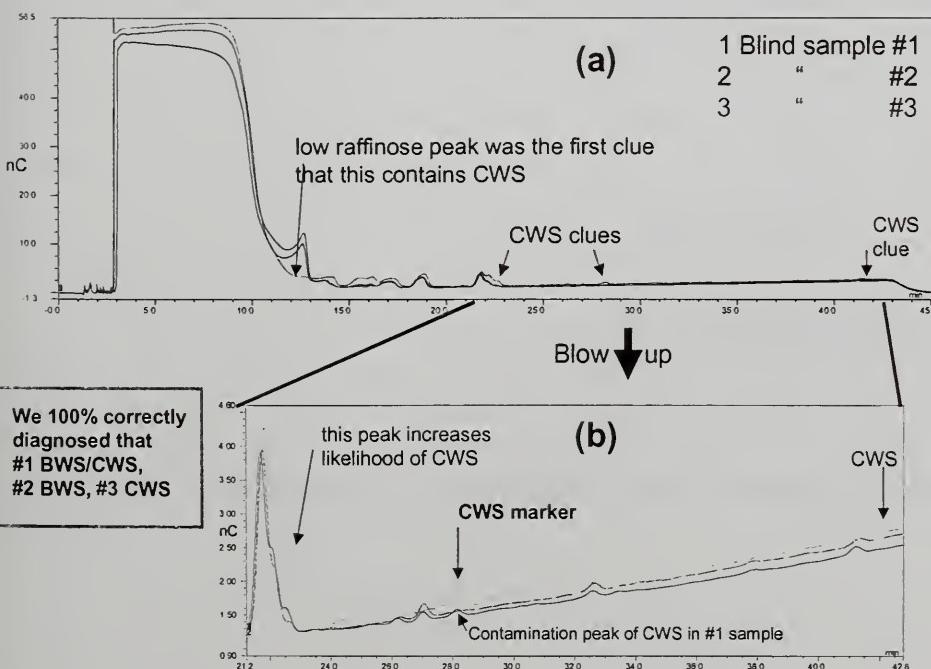


Figure 3. IC profiles to differentiate three blind samples (made from SPRI library with one containing a mix of BWS/CWS)

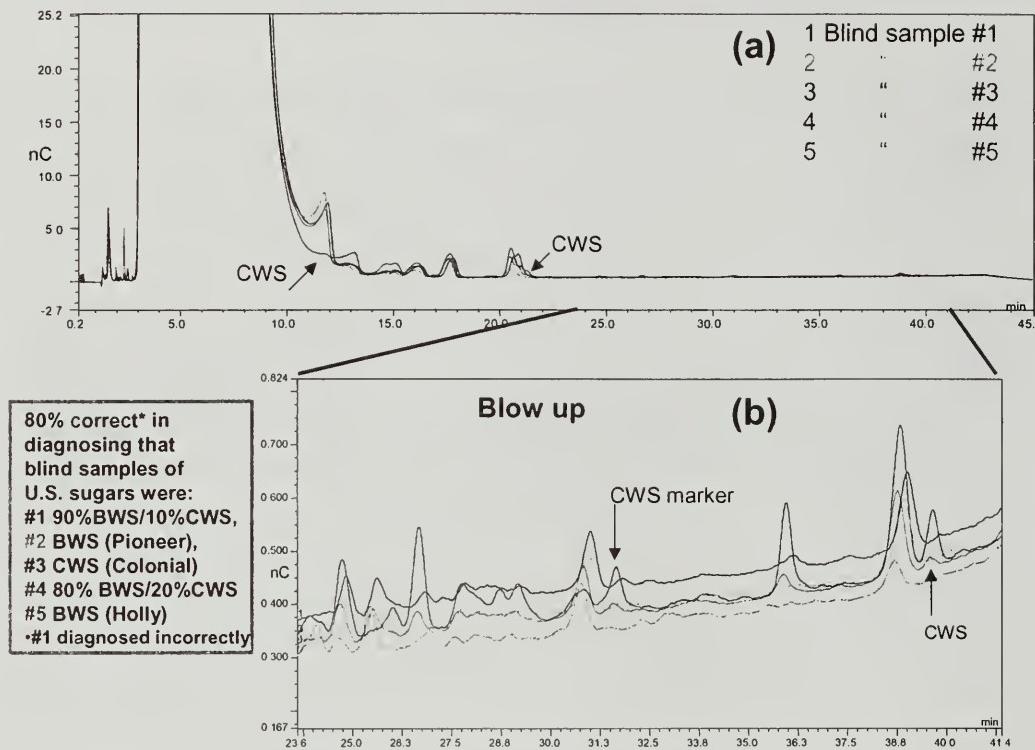


Figure 4. IC profiles of five blind samples (made from SPRI library and some BWS/CWS mixes included).

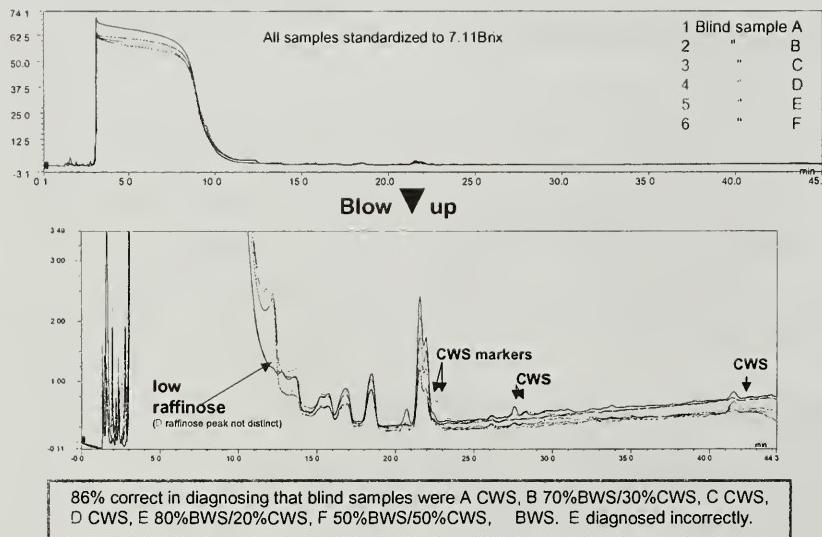


Figure 5. IC profiles of seven blind samples from Dr. Pollach in Austria.

COMPOSITION OF INDIAN PLANTATION WHITE SUGAR: COMPARISON TO INTERNATIONAL STANDARDS

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ABSTRACT

India is the second largest sugar producer in the world, but most of the sugar is produced for domestic consumption and consists of plantation white sugar, an intermediate grade of white sugar. This brief paper gives an overview of the compositional range of quality parameters in plantation white sugar compared to refined sugar and shows areas where improvement has occurred in the last decade and highlights areas where further improvements can be made.

INTRODUCTION

Depending on the statistics used, India is either the largest sugar producer in the world or the second largest. If traditional cane sugar sweeteners, such as khandsari and Gur are included, India produces the equivalent of 24-26 million tons raw value. With respect to crystal sugar production, India ranks second only to Brazil, producing 18-20 million tons of crystal sugar. Presently, about 4 million hectares of land is under sugarcane cultivation, with an average yield of 70 tonnes per hectare. The majority of sugar is direct-consumption sugar, or plantation white sugar, most of which is consumed domestically, with only 1.2 million tons exported in 2002. In view of growing sugar stocks, India is seeking to diversify its production and to export sugar. There are two avenues for India to export sugar: Begin producing raw sugar for export or improve the quality of the plantation white sugar to make it a more desirable export. Until recently, raw sugar production was not allowed by law, and it will take some time to build the raw sugar producing and exporting infrastructure. In the meantime, Indian producers have undertaken to improve the quality of plantation sugar by adopting ICUMSA methods and setting quality criteria that conform to the revised Codex Standard for Sugars 212-1999.

India has over 500 sugar mills distributed throughout the country.⁽¹⁾ The mills tend to be small, in the range of 1200-2500 tons cane ground per day, with many as small as 800-1000 tcd, and others in the range of 4000-5000 tcd. The largest mills in India are found in Uttar Pradesh state, where tonnage ground for some mills is 8000-10,000 per day.

The six major sugar producing states are:⁽¹⁾

1.	Maharashtra	159 mills
2.	Uttar Pradesh	119
3.	Karnataka	43
4.	Andhra Pradesh	41
5.	Tamil Nadu	38
6.	Gujarat	22

Most of the mills produce direct consumption sugar, also known as plantation white. It is produced from milled cane using double sulfitation for color removal. The cane is often hand cut and very clean when brought into the mill, and is usually processed fresh (see Figure 1).



Figure 1. Clean, fresh cane, tied in bundles, is brought to the factory and processed within hours of arrival.

MATERIALS AND METHODS

Plantation sugar samples were received over a period of two years from various manufacturers in India and analyzed using standard SPRI or ICUMSA procedures for pol, moisture, ash, color, glucose, fructose, total polysaccharides, starch, dextran, SO₂, turbidity, pH, sediment, and floc. They were sent to an outside commercial testing laboratory for microbiological analysis.

RESULTS AND DISCUSSION

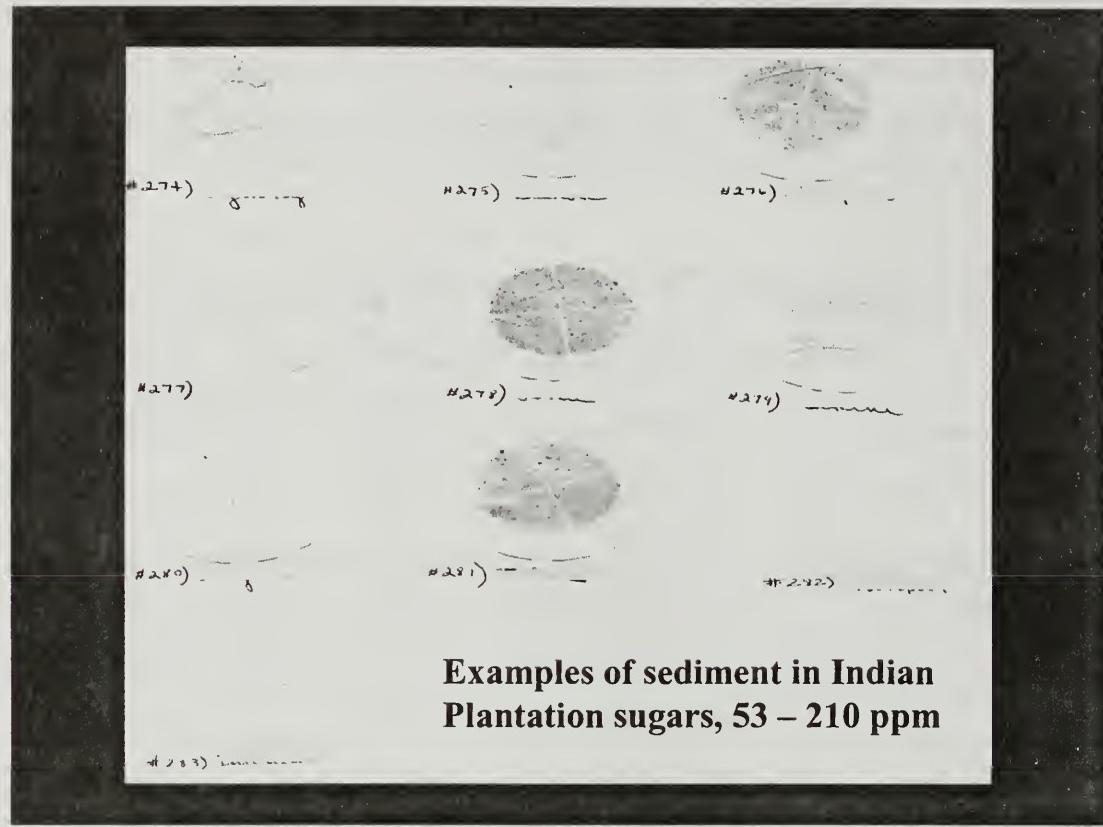
Table 1 summarizes the results for 30 to 40 sugars (not all sugars were analyzed for all parameters), and compares plantation sugar to refined cane sugar from the United States.

Table 1. Comparison of Indian plantation white sugar and refined cane sugar.

Parameter	Plantation White Sugar		Refined Cane Sugar (USA)
	Range	Mean	
Pol	99.4 - 99.8	99.6	99.9
Color, pH 7	80 - 200	136	15 - 35
Color, no pH adjustment	50 - 150	107	15 - 35
pH	5.3 - 6.1	5.7	6.2 - 6.7
Conductivity Ash%	0.03 - 0.04	0.031	0.01 - 0.03
Moisture %	0.03 - 0.04	0.035	0.01 - 0.02
Polysaccharides, ppm	150 - 260	193	68 - 200
Dextran, ppm	37 - 59	47	34 - 137 (M = 63)
Starch, ppm	29 - 170	95	30 - 50
Floccing potential	Low to moderate		Low to none
SO ₂ , ppm	8 - 15	10	Not detected
Sediment, ppm	50 - 200	117	10 - 20
Turbidity, IU	30 - 100	50	2 - 25
Turbidity, NTU	2 - 5	3.8	0 - 1.5
Glucose, %	0.01-0.013	0.010	0.005
Fructose, %	0.01-0.018	0.015	0.005
Total plate count, CFU/10g	<10 - 80	10	<10
Yeast & mold, CFU/10g	<10 - 10	<10	<10

Sediment and Turbidity.

As far as visual appearance is concerned, sediment and turbidity are perhaps the worst of the quality parameters in plantation white sugar because they tend to be high. Sediment and turbidity are closely correlated. Sediment contributes to high turbidity and unsightly material on filtration pads (See Figure 2). Sediment can range from pale beige, grey, light brown to dark brown and consists of fibers (probably from jute bags), sand, bagacillo, small flecks of iron from equipment and fine particles of soil. The quantity of material caught on the filtration membrane is sometimes quite surprising.



**Examples of sediment in Indian
Plantation sugars, 53 – 210 ppm**

Figure 2. Sediment in typical plantation sugar from a variety of Indian mills.

Polysaccharides.

Starch, dextran and total polysaccharides are either in a similar concentration range as found in refined sugar or slightly lower, as in the case of dextran. This is because plantation sugar is usually made from fresh, clean cane, so dextran, an indicator of cane deterioration, is kept to a minimum, and starch, which is concentrated in leaves and growing tip, is eliminated during the harvest.

Floc.

The floc that forms in plantation sugar does not have the same appearance as that which forms in refined sugar -- the particle sizes are smaller and it takes longer for the floc to form. If the sediment and turbidity could be brought under control, floc would probably be largely eliminated.

Other Quality Issues.

The color of plantation sugar is high, making it not suitable for some food applications, without further processing, such as for soft drink manufacture. The crystals are very large (Figure 3), causing the sugar to dissolve more slowly than desirable in some food production processes. The presence of >10 ppm SO₂ may also disqualify use in some food production. However, all indications are that color and sulfite are being reduced by improvements in the process, and the large crystals are mainly a tradition and manufacturers can learn to tailor their crystal sizes to many different applications, as is done, with great profit, in Europe and North America.



Figure 3. Plantation white sugar crystals are large (~ 1 mm) and regular.

Codex Standards vs Customer Specifications

Standards are promulgated by national bodies for health, trade and regulatory purposes and usually represent minimal quality requirements. Table 2 shows a few of the requirements in Codex Standard 212-1999 for white sugar and plantation mill white sugar (PMWS). A comparison of these minimal standards to the actual values found in plantation sugar in Table 1 shows that existing plantation sugar well exceeds those standards in most cases. The Indian draft specification ⁽²⁾ also allows 70 ppm residual SO₂ and maximum sediment of 0.1% by weight (1000 ppm).

Compared to the minimum standards set by Codex and others, specifications set by customers for purchase of sugar are usually a lot more stringent. For example, the International Society of Beverage Technologists (ISBT) are working on unified guidelines for granular sucrose, which they hope to implement around the world. A manual of these guidelines and their rationales has been issued. They use three rationales for the parameters: Process Capability, Sensory (which includes taste and appearance), and Regulatory (required by law). (See paper by Tanner and Finnerty, this volume.) Most producers eventually manufacture their product to meet customer specifications.

Table 2. Codex Standard 212-1999 for Sugars

Parameter	White Sugar	Plantation Mill White Sugar
Pol	Not less than 99.7	Not less than 99.5
Conductivity Ash, %	≤ 0.04	≤ 0.1
Invert, %	≤ 0.04	≤ 0.1
Moisture, % *	≤ 0.1	≤ 0.1
Color, ICUMSA**	≤ 60	≤ 150
SO ₂ , mg/kg (ppm)	≤ 15	≤ 70

* Determined by loss on drying

** Color measured without pH adjustment

CONCLUSIONS

In 1996, India committed to improving the quality of its sugar, with an eye to international trade, and began to study and implement ICUMSA methods. Today, the Indian sugar industry is very active in ICUMSA. Several proactive and innovative Indian sugar producers are improving their processes, even considering refining type processes to produce a better quality sugar, more in line with refined sugar.

Plantation sugar in India today routinely exceeds the minimum standards set by Codex, as well as those set by the Indian proposed draft, especially with regard to SO₂ and sediment. Work is still needed to improve sediment and turbidity levels. Plantation sugar is made from clean, hand cut cane that is processed within hours of harvest, resulting in low levels of dextran, starch and other polysaccharides, producing sugars with a low tendency to floc.

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CHANGES IN NUMBER 1 LIQUOR ON STORAGE

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ABSTRACT

There is growing interest in short term storage of cane syrup or liquor, and questions about stability and optimum conditions to prevent sucrose degradation and color formation need to be worked out. In this study, we examined changes that occurred in liquor samples under simulated storage conditions. These were compared to a model system consisting of white refined cane sugar. The changes observed in the liquors over ten days were fairly rapid and significant for all parameters analyzed. In the model system, pH and invert showed significant changes, but color remained unchanged.

INTRODUCTION

The storage of high brix sugar solutions is of potential interest to the cane sugar industry, both in the refinery and in the factory. In the factory, the storage of evaporator syrup could help to extend the season. In the refinery, storage of liquor could act as a reserve for when sugar stocks are low, or for transportation, as described at the 2002 Sugar Industry Technologists meeting.⁽¹⁾ In that report, experience on storing #1 liquor for barge transport over a period of days was presented, showing the changes that occurred in the liquor at various temperatures.

Long term storage of beet thick juice has been practiced in the beet sugar industry since 1960 as a means of extending the season.⁽²⁾ The optimum conditions for storage fall in a narrow range of temperature, pH and solids content. These conditions are not usual in the cane industry and may seem extreme. For storage of beet thick juice, a temperature below 25°C or even 10°C is recommended, as is 68-69% solids content and pH 9 or higher. Biocides are not used, although sanitary conditions are essential, and some factories place a layer of concentrated NaOH on the top of the tank to control microbial infection.⁽³⁾

Beet thick juice storage is intended for long term storage, on the order of several months, whereas cane syrup/liquor storage is intended usually for only a few days or weeks, so it may be possible that less than optimal conditions may be employed.

EXPERIMENTAL

Two Number 1 liquor samples were obtained from a refinery for the storage study. Sample aliquots of 100 ml were placed in a water bath for 2 to 10 days at 55°C. pH, color and invert were measured on day 2, 5, 7 and 10. A model system consisting of white refined cane sugar was also prepared and subjected to the same conditions as the liquor samples. Additionally, the pH of Liquor-2 was adjusted to pH 8.55 to determine if a more alkaline environment would result in less sucrose degradation, since sucrose is most stable at pH 8-9. Attempts to adjust the pH of Liquor-1 were not successful due to a very strong buffering capacity, so that the pH could only be elevated to 7.24 from 7.15. Experiments were run in duplicate.

Table 1 shows the characteristics of the liquors.

Table 1. Characteristics of liquors used for storage studies.

Parameter	Liquor-1	Liquor-2	Refined sugar
Brix	63.8	61.2	63.5
pH	7.15	7.25	7.36 *
Color	206	137	40
Glucose, %	0.108	0.135	0.01
Fructose, %	0.098	0.110	0.003
G/F ratio	1.10	1.23	3.33
Invert, %	0.206	0.245	0.013
Ash, %	0.111	0.060	0.008

*pH of refined sugar was adjusted with NaOH.

RESULTS

Table 2 shows the changes that occurred in pH. Table 3 shows the changes that occurred in color. Table 4 shows the changes that occurred in invert.

Figures 1-3 show the changes in pH, color and invert as a series of bar charts so that the various solutions can be compared. Figures 4-6 show the statistical relationships in the pH, color and invert changes.

Table 2. Changes in pH at 55°C.

Day	Liquor-1 pH 7.15	Liquor-1 pH 7.24	Liquor-2 pH 7.25	Liquor-2 pH 8.55	Refined pH 7.36
0	7.15	7.24	7.25	8.55	7.36
2	6.35	6.64	6.32	7.76	7.15
5	5.86	5.99	5.69	6.56	6.60
7	5.60	5.83	5.34	6.11	6.45
10	5.19	5.38	5.09	5.58	6.21
Total pH drop	1.96	1.86	2.16	2.97	1.15

Table 3. Changes in color at 55°C.

Day	Liquor-1 pH 7.15	Liquor-1 pH 7.24	Liquor-2 pH 7.25	Liquor-2 pH 8.55	Refined pH 7.36
0	206	228	137	137	40
2	232	251	166	222	32
5	261	273	191	258	32
7	289	291	209	274	32
10	316	315	226	291	35
% Increase	53.4	38.2	65.0	112	0

Table 4. Changes in invert at 55°C.

Day	Liquor-1 pH 7.15	Liquor-1 pH 7.24	Liquor-2 pH 7.25	Liquor-2 pH 8.55	Refined pH 7.36
0	0.206	0.201	0.245	0.229	0.013
2	0.232	0.225	0.276	0.240	0.021
5	0.333	0.292	0.403	0.274	0.043
7	0.475	0.361	0.610	0.295	0.063
10	0.674	0.568	1.023	0.458	0.150
Increase	3.3 x	2.8 x	4.2 x	2.0 x	11.5 x

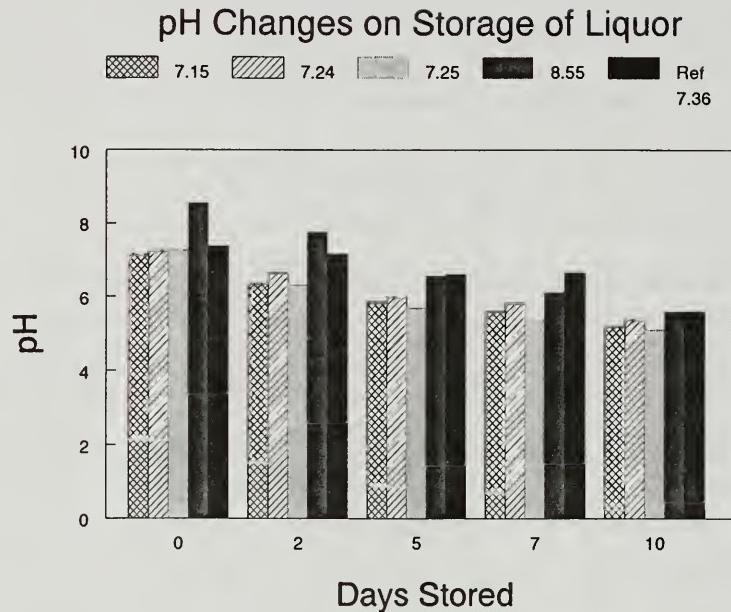


Figure 1. This graph shows the decline in pH over 10 days of storage at 55°C. The first two bars represent the same liquor, with different starting pH values, as shown in the legend. The third and fourth bars represent another liquor, with different starting pH values. The black bar represents liquor made with refined sugar.

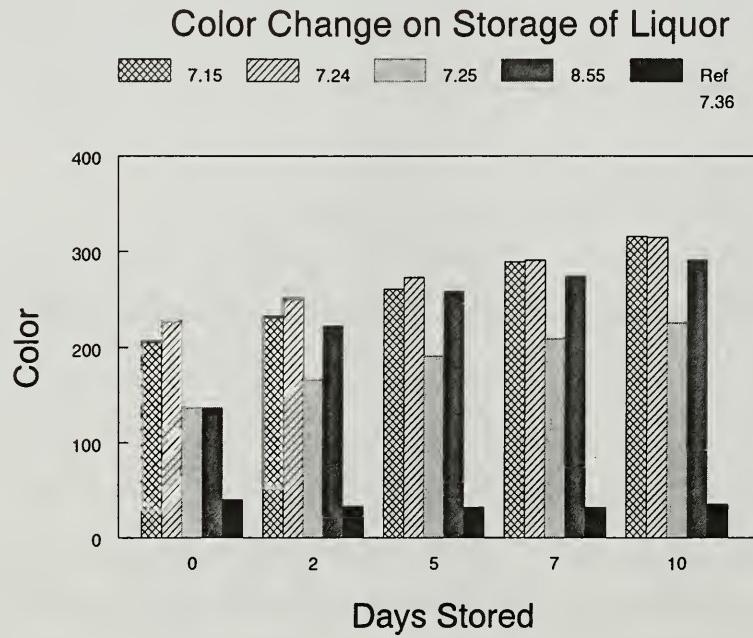


Figure 2. This graph shows the increase in color over 10 days of storage at 55°C. The first two bars represent the same liquor, with different starting pH values, as shown in the legend. The third and fourth bars represent another liquor, with different starting pH values. The black bar represents liquor made with refined sugar.

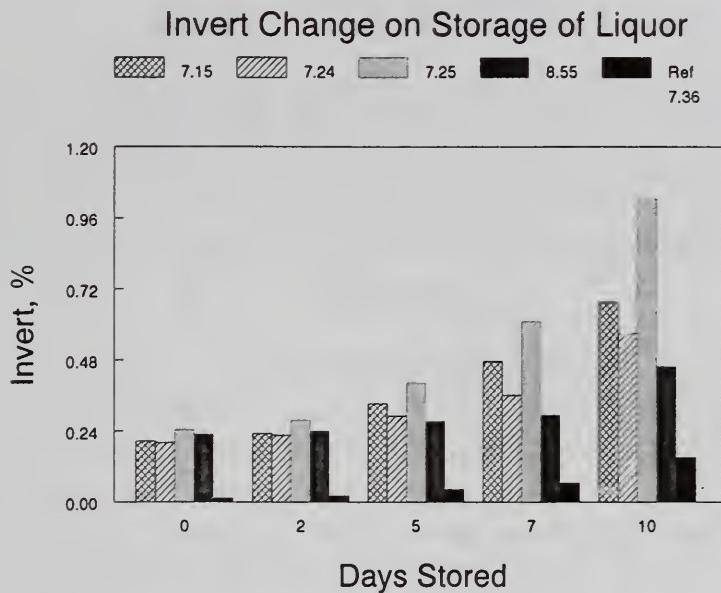


Figure 3. This graph shows the increase in invert over 10 days of storage at 55°C. The first two bars represent the same liquor, with different starting pH values, as shown in the legend. The red scale bars represent another liquor, with different starting pH values. The black bar represents liquor made with refined sugar.

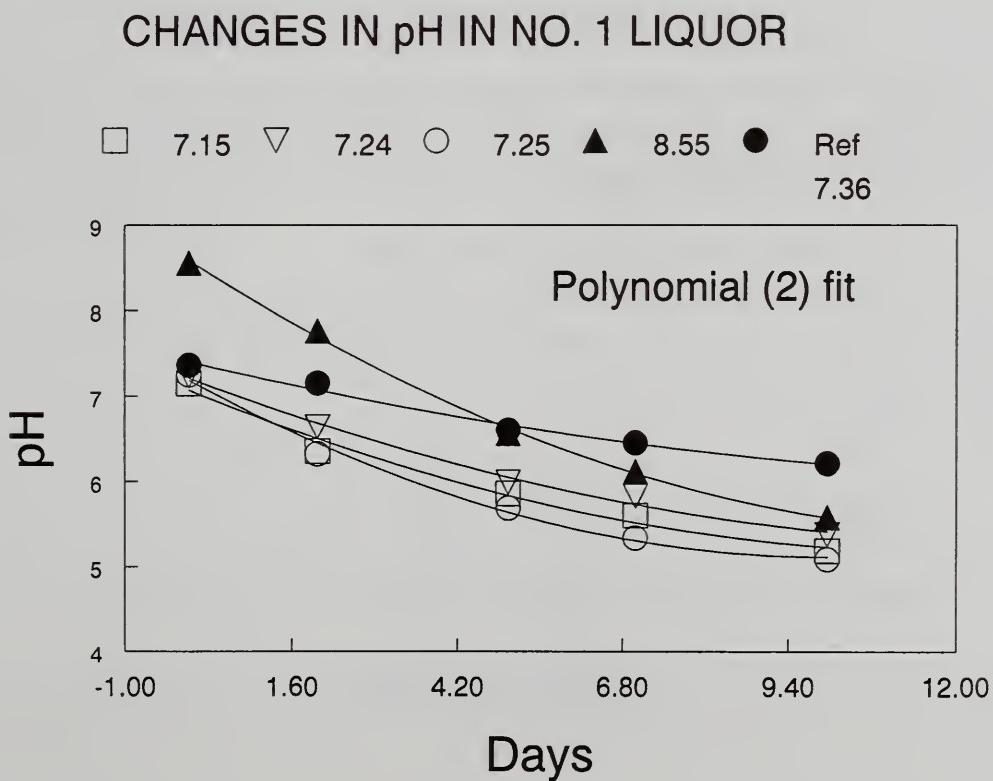


Figure 4. Changes in pH in liquors over 10 days of storage at 55°C. The fit for all pH changes is a second order polynomial with R>0.99.

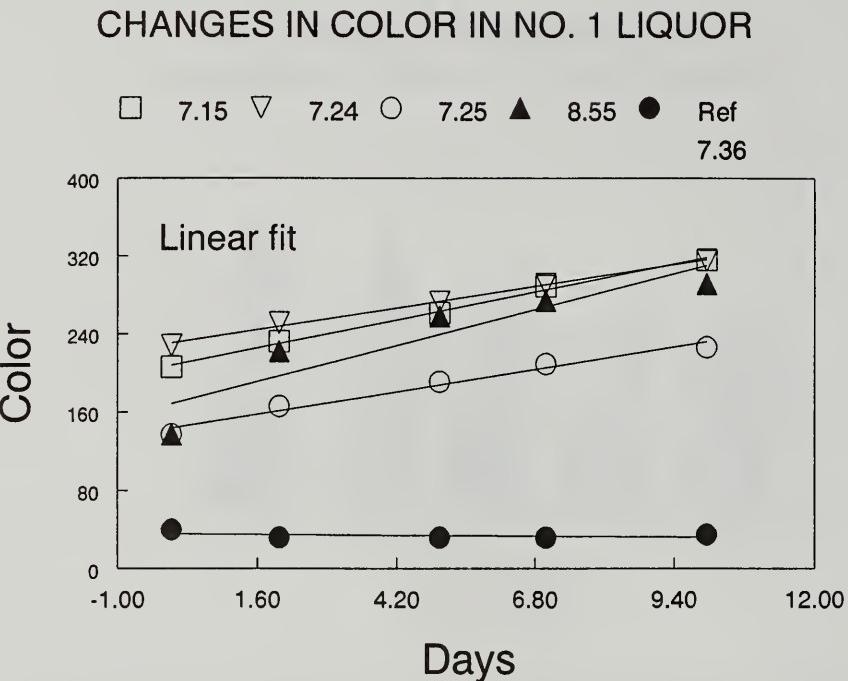


Figure 5. Changes in color in liquors over 10 days of storage at 55°C. The black circles (bottom line) represent liquor made with refined sugar, at pH 7.36.

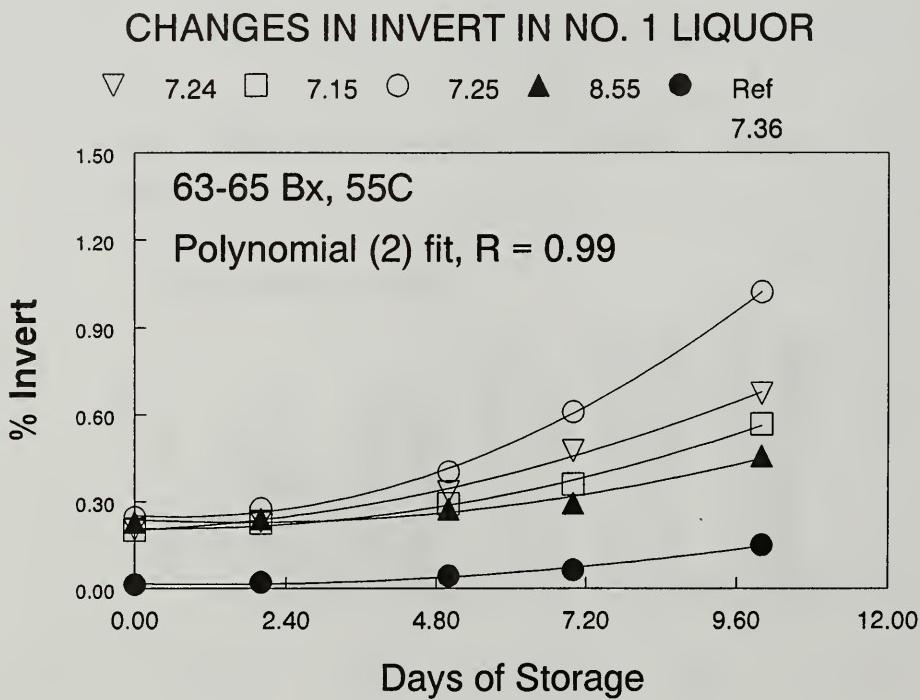


Figure 6. Changes in invert in liquors over 10 days of storage at 55°C. The black circles (bottom line) represent liquor made with refined sugar at pH 7.36. Invert changes exhibit a strong second order polynomial relationship with $R>0.98$.

DISCUSSION

This study has shown the changes that can occur in liquor under storage conditions. The changes are fairly rapid and of a significant order. We noted that changes occurred even when the liquors were at room temperature. For example, the pH of Liquor-1 when received was recorded as 7.15, lower than the pH of 7.31 noted by the refiner who provided it. Liquor-2, when received, was analyzed in SPRI to have pH 7.43, but when the analyses were started about a week later, the pH had already dropped to 7.25.

We adjusted the pH of Liquor-2 to 8.55 to see if the increased pH would stabilize the system, as sucrose is most stable around this pH. These results showed that there was no real benefit to this practice because the effect of the heat overcame the benefit of alkaline pH. Sucrose susceptibility to alkaline degradation increases exponentially as temperature is raised. Although invert levels were lower in the pH 8.55 liquor, the rate of color increase was higher, suggesting that some of the invert was consumed to make additional color.

The temperature of 55°C was chosen as representative of the average temperature a refinery liquor would reach during storage.

To summarize, invert in liquors in the pH 7.2 range increased 3-4 times; the pH 8.55 liquor invert increased only 2 times. Color increase in liquors in the pH 7.2 range was 38-65%, depending on the liquor and the pH, while color at pH 8.55 increased 112%. pH drop in the 7.2 pH range liquors was about 2 points, while the pH 8.5 liquor decreased 3 points.

The invert changes represent sucrose losses of 0.47% in Liquor-1 at pH 7.15 and 0.37% at pH 7.24; 0.78% in Liquor-2 at pH 7.25 and 0.23% at pH 8.55; and 0.14% in the refined sugar model system at pH 7.36. The sucrose losses may be slightly higher, as some of the formed invert would have been destroyed to make color.

The danger of using model systems of refined sugar to illustrate potential changes on storage are shown in this study. The liquors behaved differently, with regard to color formation, from the refined sugar model system. The refined control showed no increase in color over the 10 days, and had a 1.15 point drop in pH, from 7.36 to 6.21. Invert increased ten times, from 0.013% to 0.150%. We did not expect to see such changes in pH and invert, as sugar is supposed to be most stable in the range of pH 7-8, but again, we are seeing the influence of higher temperature. While the color remained stable, pH and invert did not. However, to all visual appearances, the refined sugar model system appeared unchanged, and the actual sucrose loss over the 10 days was in the order of 0.14%.

The results indicate that the color already present in the liquor helped to catalyze continued color reactions. This is accelerated by the higher temperature, and represents a form of alkaline degradation.

Changes in color are linear and changes in invert are polynomial, with an increasing rate of formation over time.

Previous Study

At the 2002 SIT meeting, a report was presented on storing #1 liquor for barge transport⁽¹⁾, showing the changes that occurred over time in the liquor at various temperatures. We compared our results to the results published in the SIT report, as shown in Table 5. It would appear that the results of our study fall in line with the previous study. This is also illustrated by Figure 7, which shows the SPRI data for color falling along a second order polynomial equation with a correlation of 0.9986. These results show that liquors are very sensitive to changes in pH, color and invert, even under fairly mild temperature conditions.

Table 5. Comparison of SPRI storage results to SIT-2002 storage results.

SIT-2002 samples, after 9 days:

At 38°C	1% color increase	0.3 pH drop	20% increase in invert
At 49°C	28% color increase	1.0 pH drop	33% increase in invert
At 71°C	90% color increase	1.7 pH drop	13-fold increase in invert

At SPRI, #1 liquor, after 10 days: (Average of 3 experiments, pH 7.15-7.25)

At 55°C	52% color increase	2.0 pH drop	3-fold increase in invert
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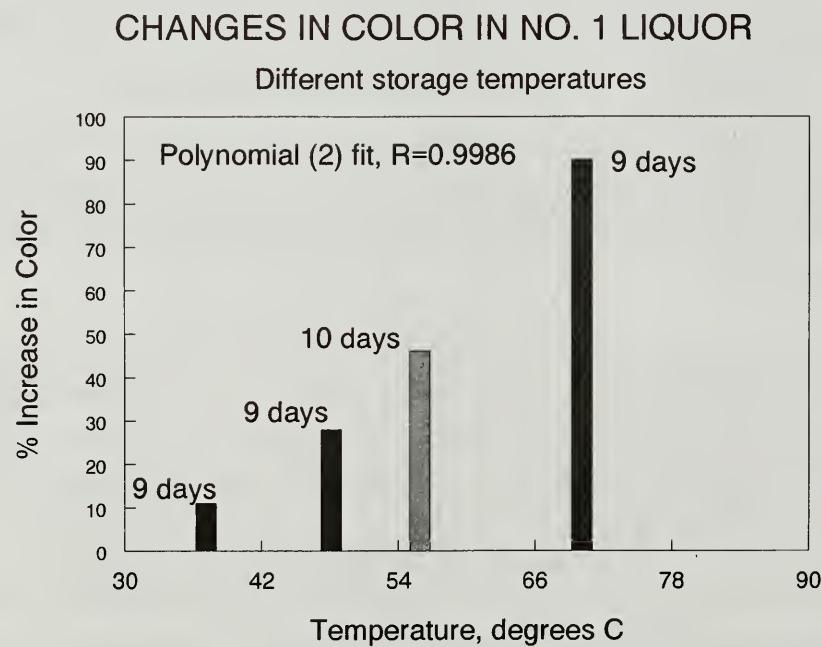


Figure 7. Increase in the color of No. 1 liquor from various sources at different storage temperatures. The 9-day samples are taken from the literature⁽¹⁾ and the 10-day sample (grey bar) is from SPRI work.

As mentioned earlier, the long term storage of beet thick juice or molasses desugaring extract is done under quite different conditions than those of this study. For microbiological and chemical control over the long term, some beet molasses desugaring extracts are stored as high as 69% solids content, pH > 10.5 and temperature < 15 °C.⁽³⁾ In most areas where cane is produced or refined, it would be very difficult to impossible to attain the optimum low temperatures, which means that the high pH levels used in beet storage would also have to be avoided, since high pH is the principal determinant of color and invert formation at elevated temperatures. However, a higher solids content probably could be attainable and would be beneficial for any (short term) storage of cane syrup or liquor.

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LABORATORY CLARIFICATION TESTS WITH NATURAL FLOCCULANTS: POTENTIAL APPLICATIONS FOR ORGANIC SUGAR PRODUCTION

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ABSTRACT

The primary objective of the clarification process in sugar production is to obtain a juice with good color and low turbidity by removing the maximum amount of the impurities (dissolved and/or in suspension). Clarification is done by adding synthetic anionic polyelectrolytes, which are not acceptable for producing organic sugar. For this reason, this research evaluated watery or mucilaginous extracts from the fruits of the plant *Cordia lutea* (Fam. Boraginaceae) and chitosan as possible flocculating agents to clarify cane juice. Two polysaccharides responsible for the flocculant properties that contribute to the removal of impurities in cane juices were found in the watery fraction of *C. lutea*, bound to an insoluble lignocellulosic portion. The general sedimentation tests on a lab scale and the conditions for using both the watery extract of *C. lutea* and chitosan as flocculating agents in cane juices are discussed in this paper.

INTRODUCTION

The clarification of cane juices is one of the most significant technological challenges in the production of organic sugar. The synthetic flocculants used to make commercial sugar need to be replaced by natural products in order to produce organic sugar.

Taking into account these criteria in the clarification of cane juices for producing organic sugar, natural chemical products (not manufactured) such as lime, natural phosphates and plant flocculants (mucilages) should be used. Other potential flocculants of natural origin used in the food industry, pharmaceuticals and for water treatment merit special attention in the evaluation

and search for the respective accreditation within the current regulations for organic products (Victoria, J.I. *et al.*, 2000).

For this reason a variety of plant materials with flocculant properties, such as the stalk of *Abelmoschus esculentus* (L.), commonly known as lady's fingers or okra, and the gums of the dye plant *Lannea coromandelica* (Anarcadiaceae) have been reported. Moreover, the clarifying property of the seeds of the drumstick or horseradish tree *Moringa oleifera* (Moringaceae) for purifying food and water, as well as their successful application in the clarification of cane juices, is well known in developing countries (Wong Sak Hoy, Y.L., 1999).

In Central America, some small cane mills use mucilaginous extracts of the bark of certain trees as flocculating agents. This product is known as *mozote* in this sugar-producing region (Chen, J.C.P.; 1995).

Another plant material that has clarifying properties is *Cordia myxa* L. (Boraginaceae), also known as Sebestens plum or Sudan teak. Its name is due to its adaptability in furniture making (Wong Sak Hoy, Y.L.; 1999).

In Colombia watery extracts of plant species such as "cadillo" (*Triumfetta lappula*) and "guácimo" (*Guazuma ulmifolia*) have traditionally been used in the clarification of cane juices for producing panela, and uncentrifuged sugar similar to Indian gur. Their use is now being evaluated in for producing organic sugar, with promising results. Nevertheless, the low availability of the plant sources for continuous production and the possible risks of inducing deforestation of the aforementioned plants have made it indispensable to explore other natural sources. As an alternative to this search for potential flocculant products, the flocculant properties of chitin, partially deacetylated chitin and chitosan were explored. These are animal waste products that have been used to remove impurities and colorants from sugarcane solutions given that they are polysaccharides with a chemical structure similar to that of the synthetic flocculants used in the sugar industry (Pan, Y. *et al.*, 2000).

With the purpose of establishing the methodologies for managing, preparing and operating conditions for natural flocculants such as chitosan and other promising plant species on a lab scale, CENICAÑA conducted a series of evaluations that indicate opportunities for clarifying cane juices with chitosan and the mucilage of *biyuyo* (*Cordia lutea* Lam.) (Fam. Boraginaceae). In addition, the chemical composition of this plant's mucilage was characterized for its use as a clarifying agent.

MATERIALS AND METHODS

General Sedimentation Tests and Juices Used

On a lab scale 380 tests were run, using equipment for testing sedimentation (SRI - Settling Testing Kit), consisting of tubes (60 mm in diameter), calibrated from 0 to 100% with a 40-cm sedimentation capacity and a total volume of approximately 1.0 L. Cane juices with an average intrinsic phosphate content of 486 ppm were used. For each clarification test, a liter of clean primary juice obtained at CENICAÑA, limed cold with whitewash (approximately 20° Baumé) was used and then heated to the desired temperature (from 70 to 97°C) when the flocculant

(synthetic or natural) was added with constant agitation (at around 5 rpm). The juice was poured at given time intervals (every 30 s) into one of the jars measuring the height of the solid-liquid interphase.

The clarification curves were compared with a synthetic polyacrylamide flocculant as control, in this case using the same conditions and rates as those employed by the sugar mills. As response variables for the best conditions for clarification, readings were taken of color, turbidity and pH of the clarified juices.

Later, under the same lab conditions, clarification tests were run with diluted industrial juices, using the rates and conditions for the natural flocculants that offered the best results in the preliminary evaluations for clean juices.

Natural Flocculants and Factors Studied

Mucilaginous solutions of *C. lutea* at 5%, chitosan at 1% in an acid medium, and as a check, a synthetic flocculant solution (at rates of 6 ppm) were used. For both natural flocculants the effects on the pH of liming the juices (from 7.0-8.0), the rates of the flocculant addition (from 3-15 ml/ L of juice) and the heating temperature of the limed juices (from 70-97°C) were studied.

A sample of the mucilaginous extract of *C. lutea* was analyzed, and by means of a series of extractions and separations done at Sugar Processing Research Institute (SPRI) in New Orleans, their composition was determined. In addition, samples of the polysaccharides isolated from the mucilage were characterized and evaluated as potential clarifying agents and removers of colorants and/or impurities present in cane juices.

RESULTS AND DISCUSSION

Effect of Liming pH

For every 10 ml of each solution of natural flocculant evaluated per liter of juice and temperature for the limed juices at 97°C, a decrease in the effectiveness of the flocculants was observed, which was directly proportional to the increase in the pH due to liming (from 7.0 to 8.0). This fact was possibly due to an increase in the concentration of Ca^{2+} ions, which caused the formation of larger and more disperse flocculated particles, resulting in slow sedimentation at the lab scale.

Effect of the Flocculant Concentration

Upon increasing the amount of added solution of natural flocculants (from 3 to 15 ml), with a pH after liming the juices of 7.5, and a heating temperature of 97°C, an increase in the effectiveness of the flocculants as clarifying agents was obtained, proportional to the increase in the addition rates of the flocculants. This was due to the fact that the increase in the rates introduced a larger concentratin of molecules of the flocculant polymer to the process, thereby ensuring the effective bonding of the flocculated particles formed in the liming stage.

Effect of the Heating Temperature

With 10 ml of each solution of the natural flocculants per liter of cane juice, limed to a pH of 7.5 and with increases in temperature from 70 to 97°C, increases in the performance of the flocculant

in the clarification were obtained, with better efficiencies obtained at a temperature of 97°C, possibly due to better coagulation associated with the reactions among the calcium, the natural phosphates, the organic acids and the natural polymeric material.

General Composition of the Mucilage of *C. lutea*

After a series of extractions and separations, it was found that the mucilaginous material contained 86% moisture and 14% solid matter. Its general composition is described in Table 1, where it can be seen that the polysaccharide fraction contained two principal components: Polysaccharide A and Polysaccharide B, associated with a lignocellulosic portion. Polysaccharide A was precipitated with ethanol at 80% and is therefore a polysaccharide typical plant cell walls. On the other hand, Polysaccharide B was characterized as a highly hydratable substance, soluble in ethanol at 80%. The proportion of polysaccharides A and B was 1:6, which means that the plant mucilage contains six times more polysaccharide B than A.

The phenol-sulfuric acid test to determine the total carbohydrate content showed that polysaccharides A and B had 43 and 84% carbohydrate, respectively. The reaction of m-hydroxybiphenyl for uronic acids showed that polysaccharide B had 2.66% uronic acids versus 0.34% for polysaccharide A.

The composition of the carbohydrates present in polysaccharides A and B in hydrolyzed samples is shown in Table 2. These results indicate that Polysaccharide A is a highly complex carbohydrate, rich in arabinose, while Polysaccharide B is a glucomannan polysaccharide associated with uronic acid and glucose (Table 2).

Table 1. General composition of the mucilaginous material of *C. lutea*.

Components	% Dry Matter
Waxes and lipids	3.22
Polysaccharide A (insoluble in ethanol 80%)	9.89
Polysaccharide B (soluble in ethanol 80%)	64.37
Total lignocellulose material	22.52
• Lignocellulose of Polysaccharide A	8.45
• Lignocellulose of Polysaccharide B	14.07

Table 2. Composition of polysaccharides A and B in mucilage of *C. lutea*.

Carbohydrates (%)	Polysaccharide A	Polysaccharide B
Rhamnose	3.4	0.9
Arabinose	13.5	1.7
Xylose	5.3	0.9
Mannose	8.1	40.4
Galactose	4.8	-
Glucose	7.1	10.3

Tests on the Activity of the Polysaccharide Fractions of *C. lutea* as Flocculant Agents

Under lab-scale conditions of liming at pH 7.5 and a juice clarification temperature of 82°C, it was observed that both the mucilage in its integral form and Polysaccharide B showed a rapid agglomeration/flocculation with some removal of color and decrease in turbidity (Tables 3 and 4). The samples of juices treated only with lime or with lime + polysaccharide A did not behave similarly in the clarification, forming small flocculates of difficult sedimentation and producing very dark juices (Tables 3 and 4). It is evident therefore that the active fraction of the mucilage is constituted principally by Polysaccharide B, but the activity of the mucilage in its integral form is comparable to the isolated fraction of Polysaccharide B, which indicates that it is not necessary to separate the polysaccharide fractions to get maximum efficiency in the cane juice clarifying stage.

Table 3. Behavior of the sedimentation of juices treated with lime and the mucilage of *C. lutea*.

Time (min.)	Only Liming	Liming + Mucilage	Liming + Poly. A	Liming + Poly. B
5	No effect	Large particles	No effect	Large particles; sediment up to 83 ml
10	No effect	Sediments up to 41 ml	No effect	Sediment up to 70 ml; clear solution
15	Fine sediment	38 ml	Formation of fine particles	60 ml
30	Sediments up to 25 ml	33 ml	Sediment up to 21 ml; juice not clear	51 ml
60	Sediments up to 23 ml	29 ml	Juice not clear	40 ml

Table 4. Color, turbidity, rate of filtration and removal of polysaccharides of clarified juices with the mucilage of *C. lutea*.

Sample Treatment	Color (IU)	Removal of Color (%)	Turbidity (IU)	Filtration	% Removal of Polysaccharides
Not treated	33,533	-	Very dark	Difficult	-
Only lime	28,298	15.6	Very dark	Difficult	12.3
Lime+mucilage	19,751	41.1	7,103	Easy	20.2
Lime+Polys. A	19,994	40.4	Very dark	Difficult	9.6
Lime+Polys. B	21,070	37.2	9,051	Easy	9.8

IU = ICUMSA units at 420 nm

Clarification Tests with Industrial Juice

Using the volume of sediments (expressed as percentages) as the criterion of effectiveness in clarification, it was confirmed that this volume decreased more than 50% in 1 minute after doing the clarifications at a temperature of 97°C and a pH of 7.5 when a solution of chitosan at 1% was used, presenting the greatest efficiency in relation to the mucilage of *C. lutea*; however, its efficiency was inferior to that of the synthetic flocculant (Figure 1).

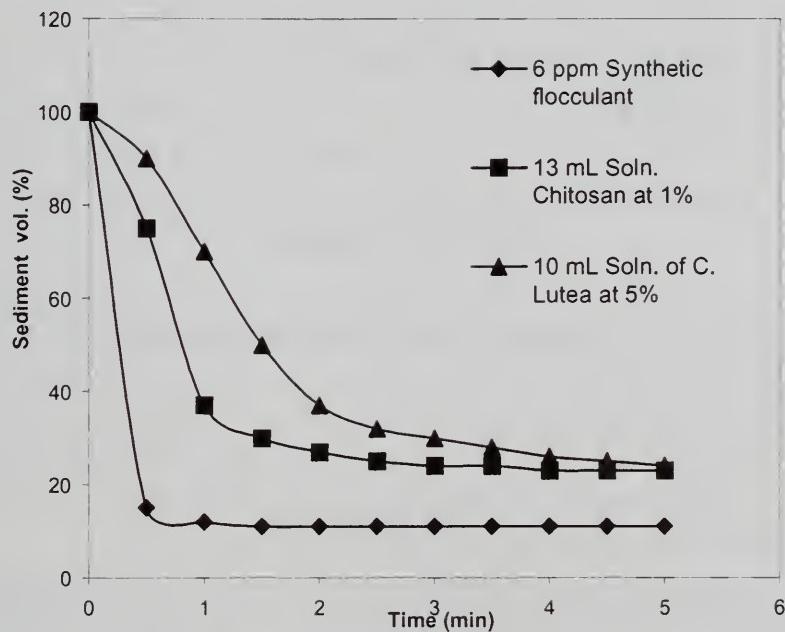


Figure 1. Clarification at pH 7.5, boiling temperature 97°C and flocculants studied for their best working rates.

Figure 1. Clarification at pH 7.5, temperature 97°C and flocculants studied for their best working rates. Sediment vol.: 6 ppm synthetic flocculant B; 13 ml Chitosan at 1%; 10 ml *C. lutea* at 5%; time (min.)

The data obtained for the response variables of the test showed that the clarified juice obtained with the solution of chitosan at 1% had the least color (12,300 ICUMSA Units), but the highest turbidity (9,400 ICUMSA Units) with respect to the other treatments (Table 5).

Table 5. Data obtained for the response variables for evaluating clarified juices with the solutions studied and synthetic flocculant as a check.

Clarification at a temperature of 97°C, pH 7.5 and different flocculants				
FLOCCULANT	Volume Sediment (%)	Final pH of Juices	Turbidity (IU) [10 ³]	Color (IU) [10 ³]
SYNTHETIC B*	23.5 A [⊗]	6.65 A	3.1 C	14.2 B
13 ml soln. Chitosan 1%	22 A	6.2 B	9.4 A	12.3 C
10 ml soln. <i>C. lutea</i> 5%	20.5 B	6.7 A	5.1 B	14.6 A

IU = ICUMSA Units at 420 nm.

* Rates of 6 ppm, 97°C and pH 7.0.

[⊗] The same letters indicate that there is no significant difference at 5% (Duncan's test).

CONCLUSIONS

- The optimal conditions for working on a lab scale for the solution of chitosan at 1% in an acid medium, were pH 7.5, 13 ml/L of cane juice, and a temperature of 97°C.
- The best conditions for operating on a lab scale for the solution or watery extract (mucilage) of *C. lutea* at 5% were pH 7.5, 10 ml/L of cane juice, and a temperature of 97°C.
- The mucilage of *C. lutea* contains two types of polysaccharides (A and B), where the most active and abundant is a glucomannan, soluble in ethanol at 80%.
- The mucilage of *C. lutea* had the advantage of producing a rapid agglomeration/flocculation during the clarification and liming of the cane juices, which permitted the easy filtration and removal of colorant material and impurities.
- The behavior of the solutions of the natural products studied in this work as natural flocculants is promising for the production of organic sugar, whose characteristics of color are less exigent than those required for white sugar. Nevertheless, other evaluations should be implemented on an industrial scale in order to determine efficiency and costs more precisely.

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THE CONCEPT OF DIFFERENT NATURAL ANTIBACTERIALS FOR THE SUGAR INDUSTRY

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ABSTRACT

During the last decade hop beta acids have been used in the sugar industry as a natural antibacterial aid, in order to control thermophilic and other Gram-positive micro-organisms. As a consequence of a very specific antibacterial action, these natural products are considered harmless to human beings, as they are regularly consumed in food. Additionally, they are biodegradable in the environment. However, micro-organisms may become insensitive to natural products after repeated application and alteration with chemicals was sometimes necessary in the past. In 2002, rosin acids were presented as a natural supplement to hop beta acids. Now palm kernel fatty acids and especially the most effective myristic acid are introduced to the sugar industry, which could again be used to displace adapted strains. After a survey of existing literature, results of laboratory trials were carried out to demonstrate the mechanism of action, as well as influences of chain length, temperature, pH etc. Further, results from full-scale factory trials during the beet campaigns 2002 and 2003 are presented. During 2002, a rosin-insensitive strain occurred in a beet extraction tower and could be eliminated by shock dosing of myristic acid. This result already stresses the importance of natural alternatives for elimination of adapted strains, to keep the level of fermentation low in every case. In 2003 satisfactory effects were achieved with myristic acid in two other *Agrana* factories. The new product is precipitable with Calcium and mainly removed from the process stream via pulp, lime sludge and molasses.

This paper won the Margaret A. Clarke Best Paper Award

INTRODUCTION

In sugar factories with hot juice extraction, such as beet sugar factories, thermophilic micro-organisms grow in the juices and cause formation of acids, gases and NO₂. *Bacillus stearothermophilus* was stated to be the only main representative of thermophilic micro-organisms in 1956 [1], but some years later a second, strictly anaerobic organism, in those days called *Clostridium thermohydrosulfuricum*, was found to be dominant in Austrian extraction towers [2]. In the meantime both micro-organisms have been renamed with respect to new taxonomic investigations [3-4]. In 1975, thermophilic bacteria growing in cane "diffusers" were mainly identified as *Bac. coagulans* and secondly as *Bac. stearothermophilus* [5]. In 1984 a coccus species was found to be the main infection in Swedish beet extraction and tentatively named *Saccharococcus thermophilus* [6]. Finally, in 1997, *Thermus* was identified as an NO₂-forming infection in the gravel bed of ion exchange columns [7].

Thermophilic micro-organisms cannot be fully suppressed by an increase of temperature, especially in beet extraction equipment without forced movement of cossettes, such as towers and DDS extraction. In the past an addition of formalin to the process stream was common practice, in order to keep the juice and the walls of the equipment as "sterile" as possible. At the end of the last century this common practice was changed to a more traditional one, because of the legal situation in some countries and voluntary decisions of some companies. In 1991, *Agrana* decided to drop the use of both formalin and dithiocarbamates and three years later the idea to use hop beta acids in the sugar industry occurred [8-9].

The natural character of sugar is often emphasised by the sugar industry - in order to contrast it with artificial sweeteners. The use of processing aids with natural origin (Figure 1) would fit in well to this natural image of sugar. As white sugar is a very purified product, the use of chemicals has no influence on the quality of sugar, but for by-products of the sugar industry, such as pressed pulp, the use of natural products could be more than an image improvement.

Under hot temperature conditions thermophilic bacteria are selected, which are mainly Gram-positive. These bacteria are sensitive to hop beta acids, according to *Shimwell* [10]. Generally speaking, substances that lower the bacterial interfacial tension to such an extent that lipoprotein membranes in the cell are disrupted will have an effect [11]. However, micro-organisms may become insensitive to such products after repeated application. In comparison to human health and the use of antibiotics, it is necessary to have different antibacterial processing aids, in order to control infections. The presentation of rosin acids at the last SPRI conference in 2002 [12] was the start of a natural antibacterial product range. Now a further group of natural acids, stemming from palm kernels (Figure 1), will be shown. Palm kernel fatty acids, which are produced by thermal splitting and fractional distillation of palm kernel oil [13], could be used in the sugar industry as a further antibacterial processing aid.

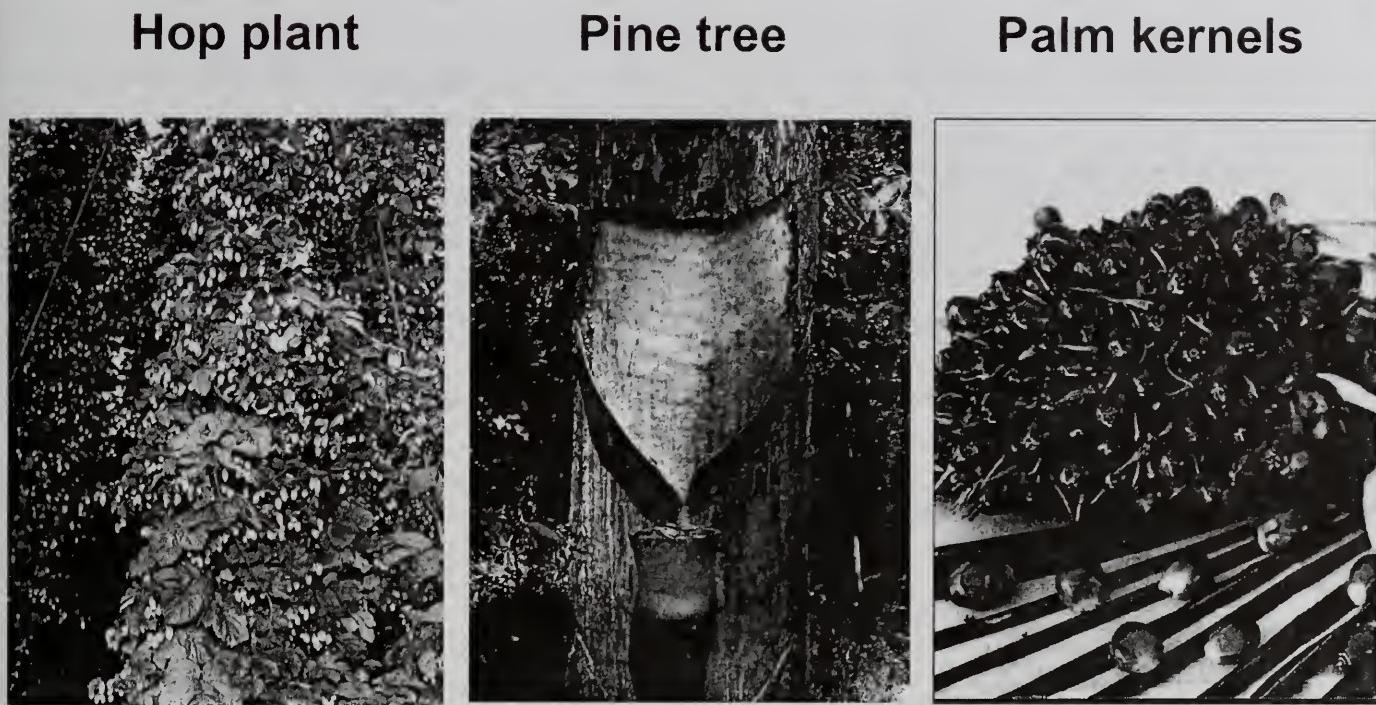


Figure 1. Natural sources of antibacterials for the sugar industry.

Palm Kernel Fatty Acids

Properties

The structure formulas of important representatives of the three types of natural antibacterials are shown in Figure 2. In contrast to the formulas of lupulone and abietic acid [14], the linear formula of myristic acid, a fatty acid with a chain length of 14 carbons, looks rather simple. The collective name "fatty acids" is less informative, because it covers compounds with very different properties and even butyric acid as the lowest one [15-17]. This acid is mainly known as a bacterial metabolite, which is unwanted in the sugar industry as it is a source of bad sugar odour [18] and causes troubles with molasses fermentation [19]. Therefore sugar technologists may associate "fatty acids" with "bad substances". At least for the literature review, the collective term "fatty acids" - as used in many titles of scientific papers - is unavoidable, but it is important to differentiate between short chain and long chain fatty acids.

Most of the palm kernel fatty acids (PKFA) are bound to glycerol and form part of the palm kernel oil, with minor amounts of free acids. Table 1 shows some information for the three important fatty acids, such as chain length, systematic name, formula, melting point, solubility and relative distribution in palm kernel oil [20]. These acids are commercially available as powdered products with high purity and are nearly insoluble in water, but soluble in ethanol. For sugar factories an aqueous potassium salt solution - in case of fatty acids corresponding to a soft soap solution - will be the most convenient variant of application.

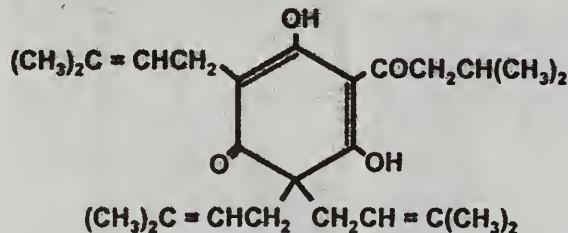
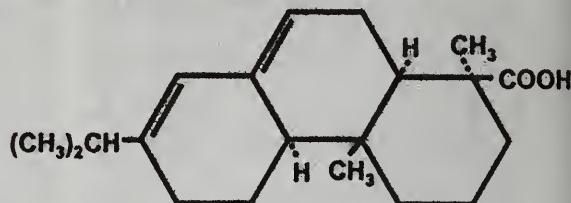
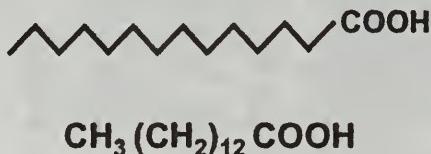
Hop plant: Lupulone**Pine tree: Abietic acid****Palm kernels: Myristic acid**

Figure 2. Effective substances from different natural sources.

Table 1. Main saturated fatty acids from palm kernel oil.

Properties	Lauric acid	Myristic acid	Palmitic acid
Chain length	C ₁₂	C ₁₄	C ₁₆
Syst. name of acid	Dodecanoic	Tetradecanoic	Hexadecanoic
Formula	CH ₃ (CH ₂) ₁₀ COOH	CH ₃ (CH ₂) ₁₂ COOH	CH ₃ (CH ₂) ₁₄ COOH
Melting point (°C)	44	54	63
Solubility (water 60°C)	0.009 %	0.003 %	0.001 %
g / 100 g PKFA	40 – 42	14 – 18	6 – 10
Other sources	coconut oil	coconut oil	palm oil

Reports about antimicrobial effects of fatty acids

A large number of reports about antimicrobial effects of fatty acids have been published and the references in this experimental paper can only be exemplary. In 1935, a toxic effect on acid-resistant bacilli was found for capric acid, in contrast to results for myristic, palmitic and stearic acid [21]. About ten years later the antimicrobial effect of fatty acids was studied in connection with soaps and skin disinfection [22]. In 1952 a maximum of effect was reported for lauric acid

[23]. A review paper of Nieman [24] dealt with the effect of trace amounts of fatty acids on bacteria on the one hand and a growth stimulation by fatty acids on the other hand. As an obvious consequence of several findings at that time, a subchapter "fatty acids" was included under "disinfection and sterilisation" in the 3rd edition of Ullmanns encyclopaedia of technical chemistry in 1954 [25]. Apparently less progress was achieved in practice, since this subchapter was considerably shortened in the 4th edition in 1975 [26] and fully dropped in the 5th edition in 1987. In spite of this negative trend, Kabara and co-workers published a series of papers about fatty acids and derivatives, e.g. [27-31], with emphasis on lauryl-monoglyceride, commercialised as Lauricidin®. In the last decade the inhibition of *Listeria* by fatty acids and monoglycerides was studied [32]. The antiviral and antibacterial activity of fatty acids and monoglycerides and the disintegration of membranes was described in patents in connection with human health [33-34]. Recently the inhibition of *Bacillus stearothermophilus* spores by free fatty acids has been examined [35]. A reference strain of this organism, proposed for evaluation of heat treatment in the canning industry, was influenced by free fatty acids from fat duck-liver preserves, and this effect contributed to the particular preserving process.

No other papers have been found that deal with the effect of fatty acids on thermophilic micro-organisms - especially with consideration of the sugar industry - and therefore a patent has been filed [36], claiming a better effect of fatty acids at high temperatures. The selection of Gram-positive micro-organisms under hot temperature conditions makes an effect very likely. Additionally - as the title of this paper suggests - it is possible to use other natural antibacterials - such as hop beta acids or rosin acids - when strain adaptation occurs.

MATERIALS AND METHODS

The main part of the laboratory trials was carried out in the same way as already reported [12]. Sterile glass vessels with pH recording and slow magnetic stirring at 65°C were used. A liquid nutrient was inoculated mainly with frozen raw juice and exceptionally with pure strains of *Geobacillus stearothermophilus* and *Thermoanaerobacter thermohydrosulphuricus*. Since products must be effective in any case with any strain, the simple method of inoculation with frozen raw juice was preferred and it proved very reproducible, but will always end up in aerobic cultures. There was a parallelism between raw-juice inoculated laboratory trials and full scale trials in the beet campaign.

After development of micro-organisms, visible as a recorded drop in pH down to 6.2 - which is close to normal pH of raw juice - fatty acids or related products were added by subsequent additions (Figure 3). Products were mainly dissolved in ethanol, but alternatively aqueous potassium soap solutions were used. In this example, the pH drop stopped immediately after the third addition. A minimum effective concentration was calculated at this point of effect. The period of inhibition is visible from pH recording and will be given as tabled results. Sometimes - in case of a short period of inhibition - the effect of further dosing was checked. The information from pH recording was completed by other measurements, such as optical density, slide culture method, bacterial staining and determination of glucose, fructose and lactic acid. Detailed information about materials and methods is given in the appendix.

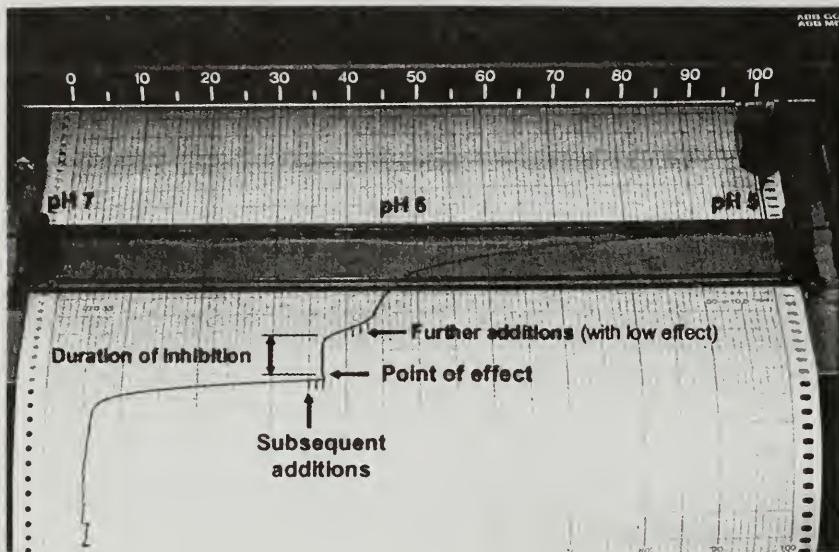


Figure 3. Laboratory trials with pH recording and subsequent product addition.

RESULTS

Results from Laboratory Trials

Influence of chain length

Typical results for two fatty acids are shown as original records in Figure 4. At 65°C, with raw juice inoculation and stepwise addition of product, palmitic acid was effective at 6 mg/L, but only for 1.5 hours. Further dosing did not fully inhibit the pH drop but caused only a slight lowering of the drop. Myristic acid was effective at the same level of 6 mg/L, but for a very long time and even overnight. A similar difference was found for hop beta acids and rosin acids in the past [12], but now the two products differ simply by two CH₂-groups. It seems that the solubility of the product in an aqueous medium is the important factor. With type strain DSM22 palmitic acid was effective for 4 - 5 hours, but a second drop in pH could not be stopped, comparable to the left diagram. Acids with chain lengths lower than C14 show a long-time-effect, but at a higher concentration. No effect was achieved with addition of solid products to thermophilic cultures.

Effects on thermophilic bacteria

A slide culture method [37] was used to demonstrate that living cells are killed shortly after dosing of myristic acid (Figure 5). The method is suitable for bacillus cultures and the micro-colonies, visible in the left picture, are counted and reported as a mean of 25 replicates. As the liquid culture is diluted at least 1:40 with liquid agar during preparation, the myristic acid is not effective during micro-agar incubation, but is effective within a few minutes in the liquid culture under investigation.

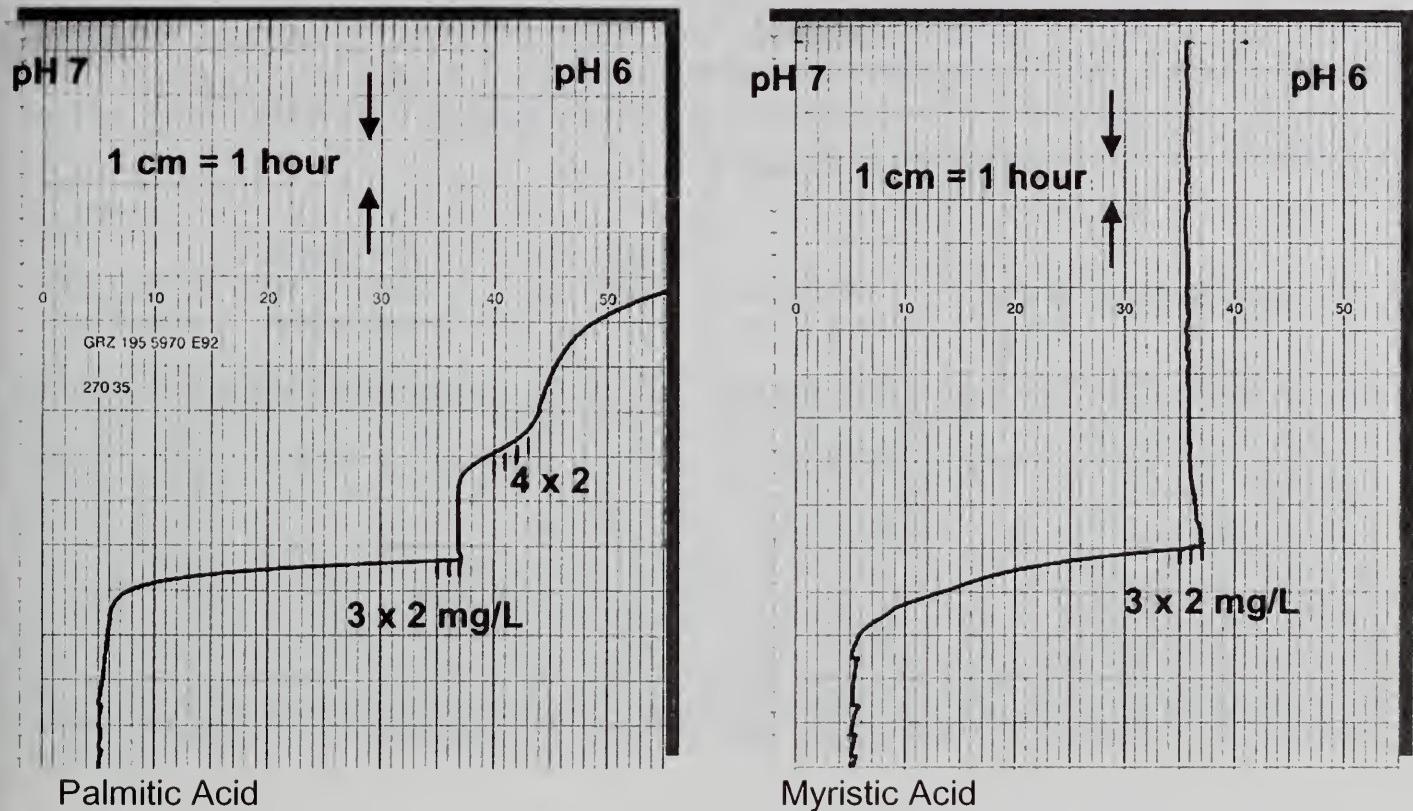


Figure 4. Comparison between palmitic and myristic acid.

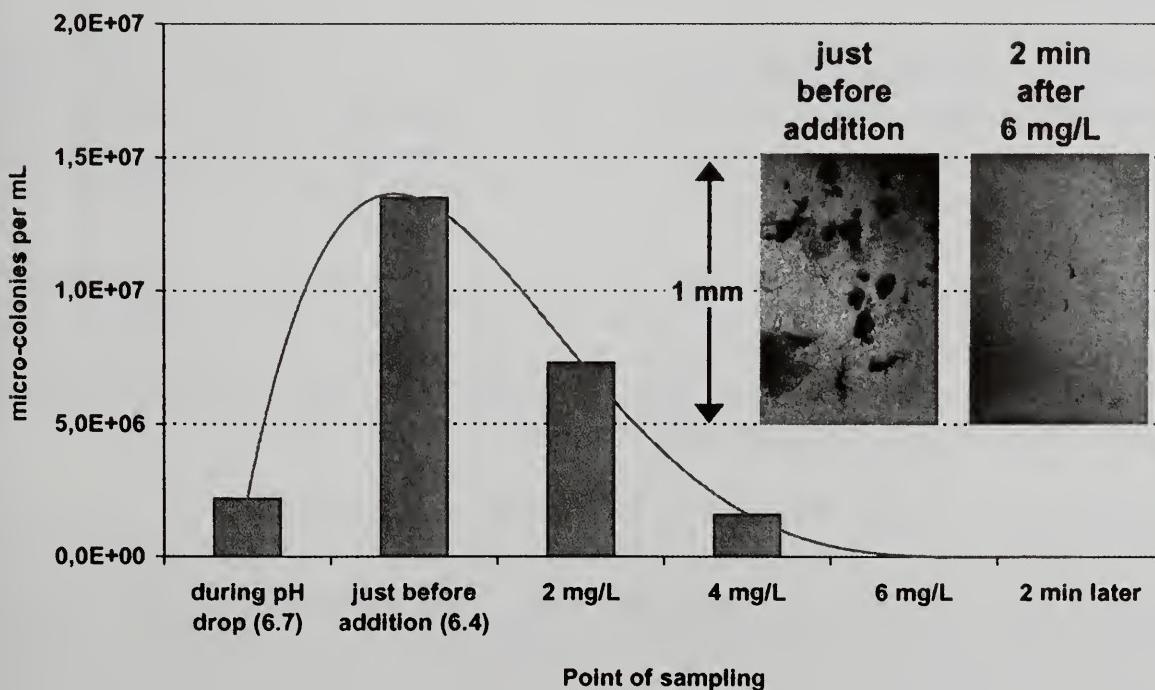


Figure 5. Slide culture method with additions of myristic acid.

The Congo red/blue staining method [38] was used to demonstrate a rapid lysis of thermophilic bacteria after addition of myristic acid (Figure 6). With this method, living cells are shown white on blue background and dead cells are blue on blue background - with low contrast. A lessening of rods is visible after product addition and finally, 4 min after a constant pH, no rods are visible in this trial. This period of lysis was confirmed in a lot of repetitions and the observation of lysis was already reported for rosin acids in the past [12].

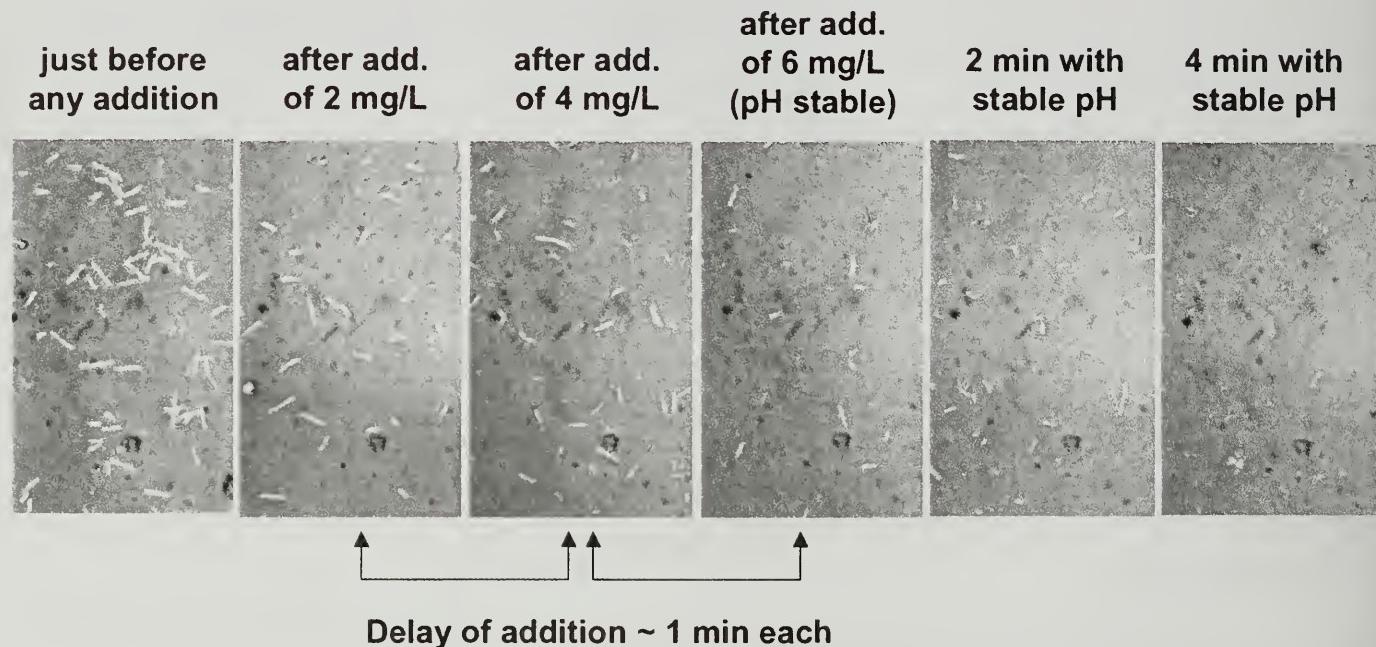


Figure 6. Lysis of thermophilic bacteria after myristic acid addition.

A heavy decrease of optical density after myristic acid addition (Figure 7) confirmed the observation obtained by the bacterial staining method. There is agreement with statements in the literature: As a consequence of a serious lowering of the bacterial interfacial tension, lipoprotein membrane disruption, leakage, disorganisation and cellular lysis was found [11][33-34].

Furthermore, after a drop in glucose due to bacterial uptake from the medium and decomposition to acid, an increase of both monosaccharides is visible. After myristic acid addition, a lysis of cells leads to a release of invertase to the medium and a parallel increase of both, glucose and fructose. The invertase is active for some hours in the batch trial, but this non-reproducing enzyme would be rinsed out in a continuous beet extraction.

The release of cell-bound invertase was again observed with the strict anaerobic, H₂S-producing organism *Thermoanaerobacter thermohydrosulfuricus*. On account of iron sulphide precipitation, it was not possible to follow up growth and lysis of the type strain DSM567 at 65°C by optical density measurements. At the beginning a parallel uptake of low amounts of glucose and fructose, stemming from heat sterilisation, is visible (Figure 8). After myristic acid addition in 4 steps of 2 mg/L, up to an effective concentration of 8 mg/L, the acid formation and pH drop stopped, but the enzymes from disintegrated cells were still active and invertase was released to the medium, similar to the aerobic strain DSM22. The natural product myristic acid doesn't attack enzymes, but only propagating bacterial cells.

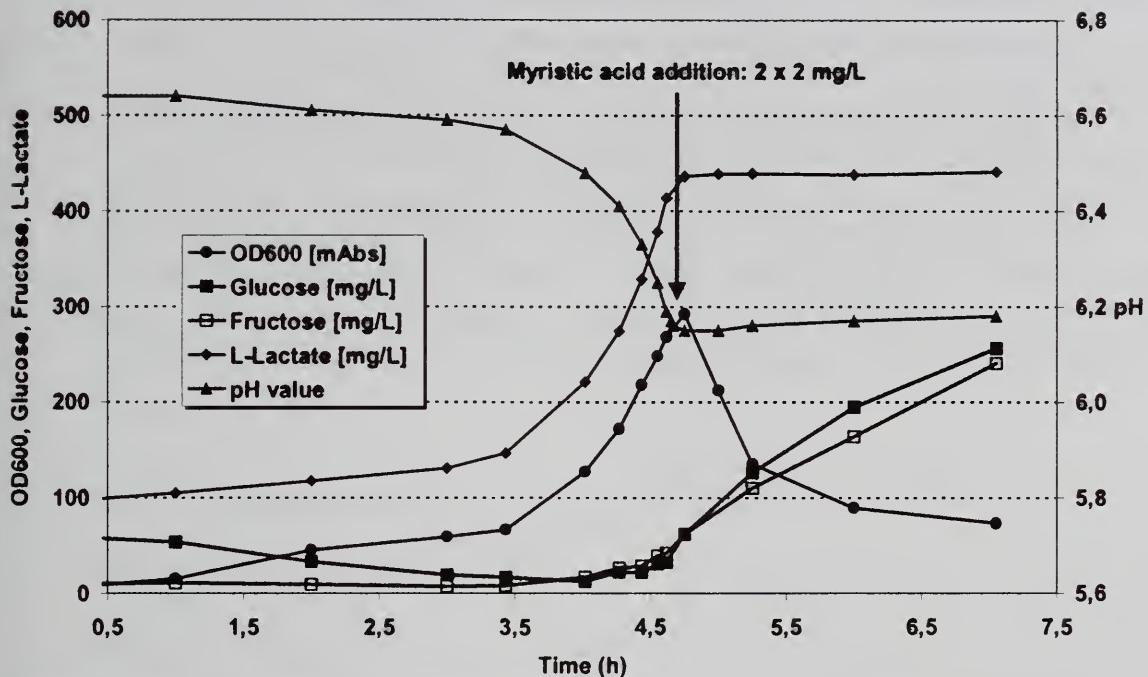


Figure 7. Optical density and further parameters of a *Geobacillus stearothermophilus* culture (DSM 22, 65°C).

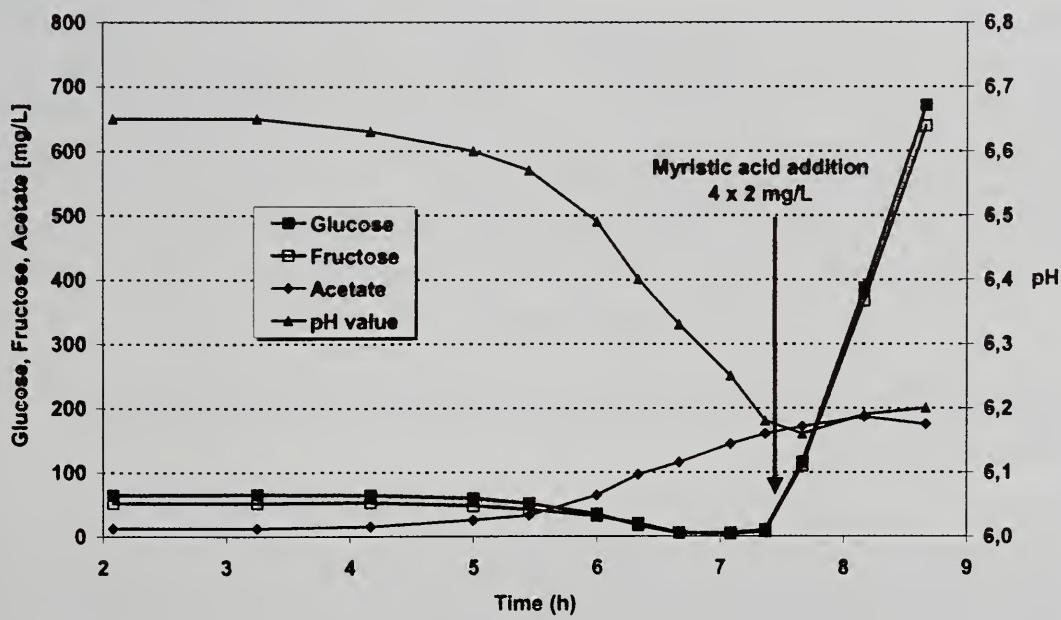


Figure 8. Release of cell-bound invertase after myristic acid addition to a culture of strain DSM567 (*Thermoanaerobacter thermohydrosulfuricus*).

Results from a further trial with some theoretical background are shown in Figure 9, with strain *Geobacillus stearothermophilus* H5-65 from the collection of Hollaus [39], cultivated at 65°C. This strain excretes invertase to the medium during growth. Glucose from sucrose splitting outside the cell is transported into the cell and converted to acid, whereas fructose remains unfermented in the medium. The strain doesn't transport fructose actively into the cell [40]. After addition of myristic acid and lysis of cells, the metabolism of glucose to acid is blocked, but due to a release of invertase to the medium a parallel formation of glucose plus fructose from sucrose is now visible. This result contributes to the understanding of monosaccharide uptake and monosaccharide formation by thermophilic bacteria, which is connected with the membrane barrier and not with extra-cellular polysaccharide formation. Polysaccharide formation is unknown for thermophiles [41] and especially unknown at temperatures above 60°C [5].

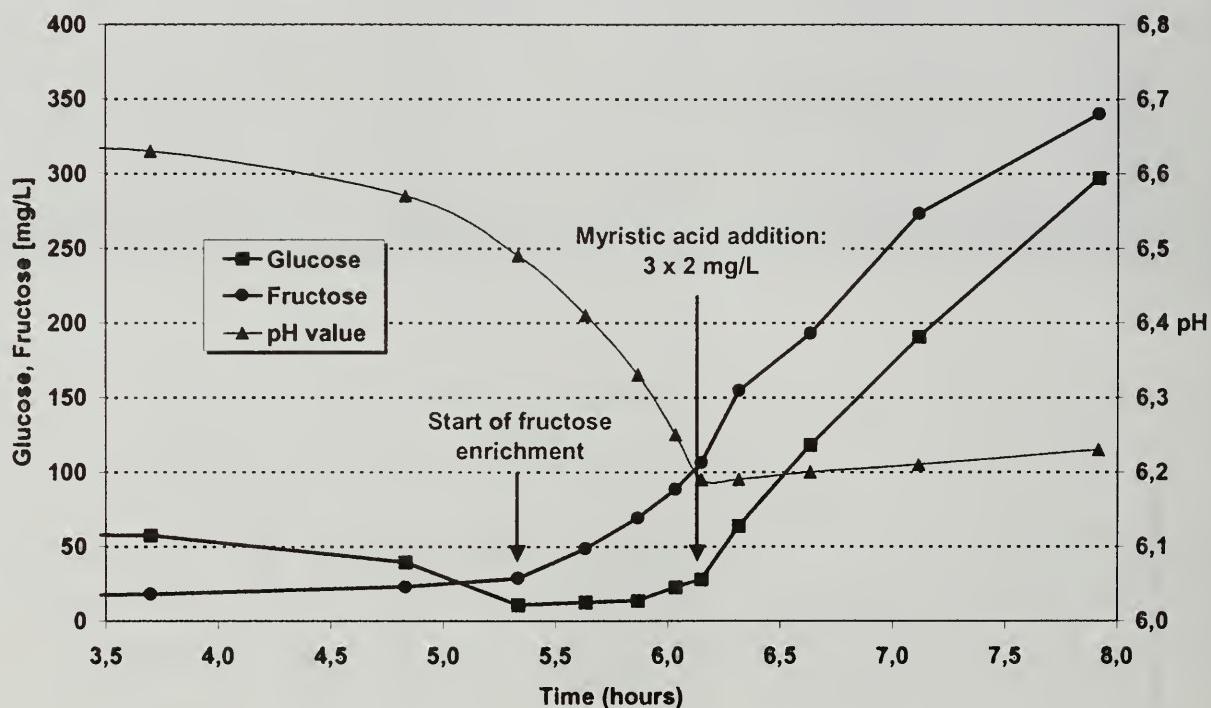


Figure 9. Influence of myristic acid on glucose enrichment in a *Geobacillus stearothermophilus* culture (H5-65, 65°C).

The last trial of this laboratory series was inoculated with raw juice (Figure 10). Optical density and L-Lactate with results similar to type strain DSM22 were not plotted here. Glucose was decomposed by this impure culture in the starting phase. Later glucose and fructose were formed in parallel in the growing culture, in contrast to the pure strain trials. After addition of myristic acid up to a concentration of 4 mg/L, both glucose formation and fructose formation were stopped. The different behaviour between pure strain trials and impure cultures may be caused by different invertase activity at 65°C and by two or more strains, growing in the impure culture. There is a parallelism between laboratory trials with raw juice inoculation like this on the one hand, and full-scale trials on the other hand, as a drop in glucose after dosing of myristic acid was often observed in full-scale trials.

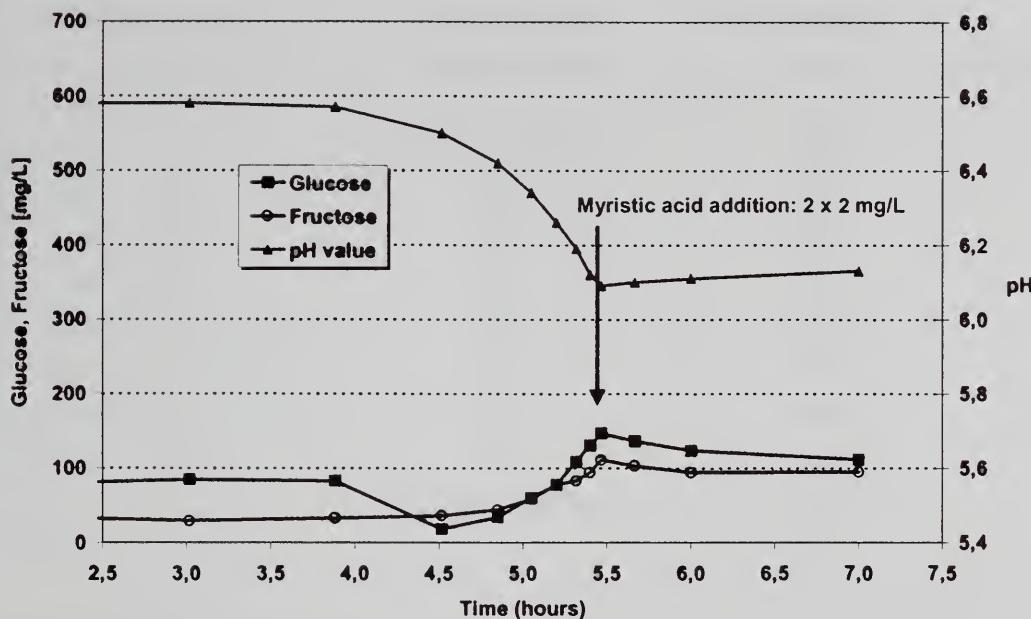


Figure 10. Effect of myristic acid addition on the course of glucose and fructose in a trial with raw juice inoculation.

Screening of fatty acids and related products

A screening study with different fatty acids in steps of 10 mg/L, was carried out with pH drop evaluation (Table 2). Acids with 20 carbons or longer were not effective. Those with 16 to 18 carbons had only a short effect, even in trials with pure strains. All acids with 14 carbons or less had a long-time effect, but the minimum effective concentration increased with shorter length of chain. Sorbic acid is widely used to preserve food, but it had no effect on thermophilic bacteria. No advantage was found for the unsaturated oleic acid over stearic acid. A maximum of effect was often reported for lauric acid in case of mesophilic micro-organisms [23] [27]. As long chain acids are more soluble at 65°C, the shift of the maximum for thermophiles - from lauric to myristic acid - is understandable. For further trials the availability of pure fatty acids from natural sources was put to the foreground, but a short side-look to related compounds will be shown.

Stearyl alcohol and some myristyl esters had no effect, because their hydrophilic group is blocked or obviously too weak (Table 3). But the effect of myristyl alcohol was equal to myristic acid and demonstrates that surface activity and not the carboxylic group is relevant. Inhibiting properties of fatty alcohols are already known [27], but the possibility to use water soluble soaps will be advantageous for acids. The mono-ester of glycerol and lauric acid - with the trademark Lauricidin® - had the same effect as lauric acid and the production of a glycerol-monoester wouldn't pay back. With respect to the requirements of the sugar industry and the natural concept of this paper, no further chemicals were studied.

Table 2. Screening of fatty acids at 65°C with additions of 10 mg/L

Fatty acid (dissolved in ethanol)	Symbol	Min. effective conc. (mg/L)	Duration of effect (hours)
Arachinic acid	C20	no effect with 150	
Stearic acid	C18	10	1.5
Oleic acid	C18:1	10	0.75
Palmitic acid	C16	10	1.5
Myristic acid	C14	10	> 9
Lauric acid	C12	30	> 6
Undecanoic acid	C11	40	> 8
Decanoic acid	C10	150	> 9
Sorbic acid	C6:2	no effect with 150	

Table 3. Related products of fatty acids with additions of 10 mg/L

Product dissolved in ethanol	Min. effective conc. (mg/L)	Duration of effect (hours)
Stearyl alcohol	no effect with 150	
Propyl myristate	no effect with 150	
Ethyl myristate	no effect with 150	
Methyl myristate	110	1
Myristyl alcohol	10	> 13
rac-Glycerol 1-laurate	30	> 11

At the beginning, lauric acid was not further considered since myristic acid showed a better effect in all trials, either with coarse steps of 10 or with fine steps of 2 mg/L. But later special effects were found (Table 4). A mixture with a mass ratio of 1:1 is expected to be effective at 12 mg/L, which is the arithmetical mean between 6 for myristic and 18 for lauric acid. But the mixture was effective at 8 mg/L, close to myristic acid. This observation can be studied further and might be useful for formulation of products from palm kernel fatty acids. Therefore the fate of lauric acid residues will be studied as well, but it was not possible to check all combinations in full scale trials till now.

Table 4. Effect of mixtures 1:1 (mass) of myristic and lauric acid.

Product addition: Σ 2 mg/L per step	Min. effective conc. (mg/L)	Duration of effect (hours)
Myristic acid	6	8
Myristic acid	6	7
Myristic + lauric acid	8	8.5
Myristic + lauric acid	8	9
Potassium myristate + laurate	8	5
Potassium myristate + laurate	8	7
Potassium myristate + laurate	8	6.5
Lauric acid	18	> 12
Lauric acid	18	> 11.5
Lauric acid	18	11.5

Effect of temperature and pH

The importance of temperature was studied in further trials. A nutrient, inoculated with raw juice, was incubated at different temperatures from 35°C up to 65°C (Table 5). The lowest temperature is typical for incubation of mesophilic organisms, while the highest one is widely used for thermophiles. A good effect for both, myristic acid and potassium myristate, is visible at 65° and 55°C. No or nearly no effect is visible at lower temperatures. Sometimes little effect was achieved at 45°C, dependent on the developing bacteria. But the two extreme temperatures, 35° and 65°C, were always reproducible. The observation stresses the importance of a pre-selection of sensitive micro-organisms by high temperature conditions. A failure of effect with fatty acids at mesophilic temperature conditions is to be expected, if no other selective criterion can be found. Promising results from trials with sensitive pure strains will never withstand conditions of practice.

Table 5. Temperature dependent trials with additions of 10 mg/L

Product	°C	Min. effective conc. (mg/L)	Duration of effect (hours)
Myristic acid	65	10	> 9
Myristic acid	55	10	> 5
Myristic acid	45	no effect with 150	
Myristic acid	35	no effect with 150	
Potassium myristate	65	10	> 12
Potassium myristate	55	10	4
Potassium myristate	45	150	3
Potassium myristate	35	no effect with 150	
Myristic acid rep.	65	10	> 13
Myristic acid rep.	35	no effect with 150	

A further restriction to conditions of beet and cane extraction is dependent on pH value. In Figure 11 trials with different pH range are shown for hop beta acids, rosin acids and the new myristic acid, with pH values measured at 65°C at the point of addition. Myristic acid shows a larger drop in effect at pH 7 than rosin acids and especially hop beta acids. The latter seem to be the most universal antibacterial aids, which can be used even in alkaline juices, according to earlier reports [9-10]. The great drop in effect at pH 7, found for myristic acid in several trials, must be considered for laboratory trials with normal laboratory nutrients and growth inhibition at pH values close to 7, which is different from that of natural beet and cane juices.

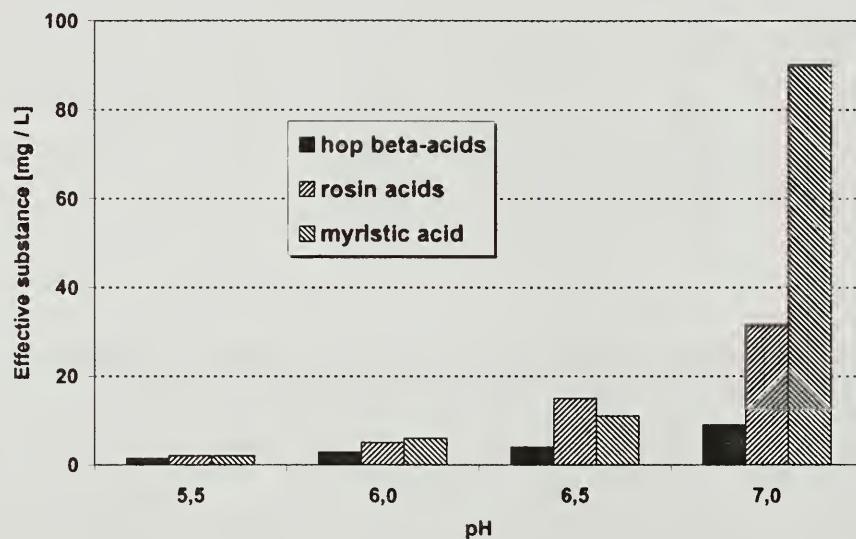


Figure 11. Influence of nutrient pH on the effects of natural antibacterials.

Results from Full Scale Trials in 2002

The first full scale trials have been carried out at the sugar factory *Tulln* during the campaign 2002 (Figure 12). They were kept short in order to get some first ideas about the fate of the fatty acid. Three manual shocks per daytime were applied, with a delay of 4 hours. For myristic acid this corresponded to a dosing of 20 g/t of daytime-beet or 10 g/t of daily beet. A repetition is represented by one day and there was a rotating system of days with a) blanks, b) rosin acids, c) hop beta acids and d) the new myristic acid. For a relative comparison of three products this rotation system proved well. During the period of these 19 trials (days) in November 2002 rosin acids were not effective - after two years of success. But for both, hop beta acids and myristic acid, a distinct difference to the mean of the blanks is visible.

Results from the last period of the campaign 2002 in *Tulln* are shown in Figure 13. A period with high levels of lactic acid, caused by a fault of the dosing pump, represents the blank. With the new myristic acid and with dosing around the clock it was possible to re-establish an effect of rosin acids, which had - as already demonstrated - not always been effective during this campaign.

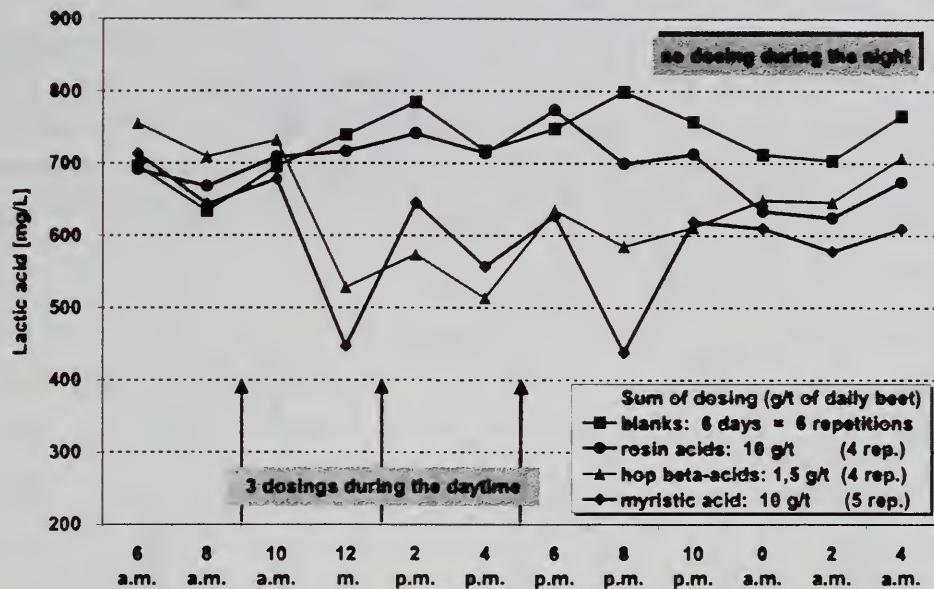


Figure 12. First full scale trials at Tulln during the beet campaign 2002.

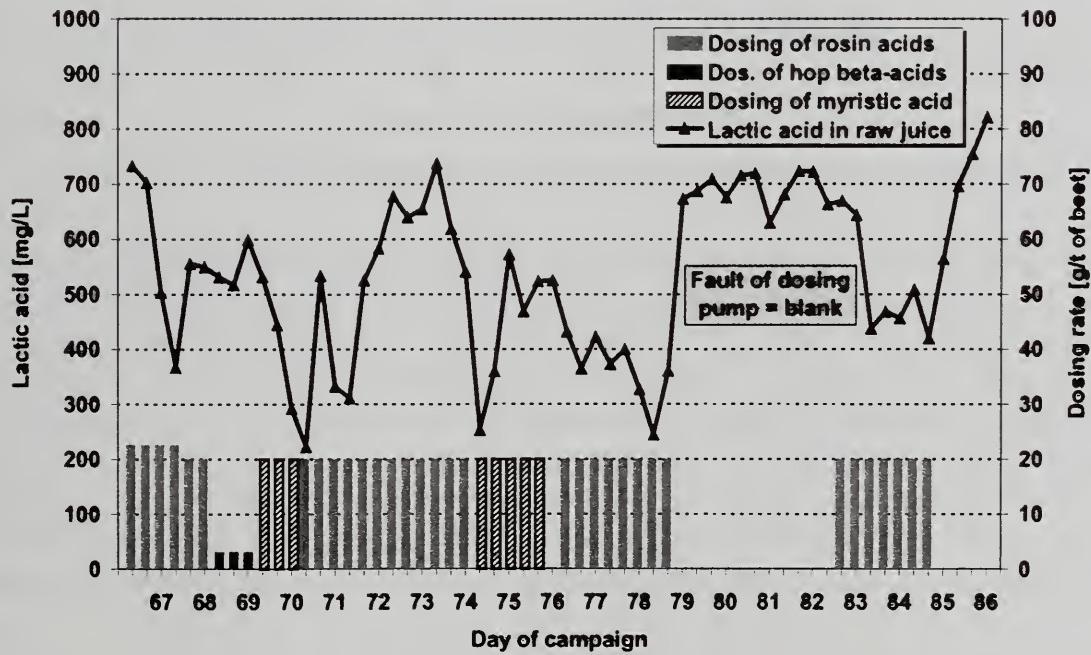


Figure 13. Re-establishing an effect of rosin acids at Tulln (2002).

Results from Full Scale Trials in 2003

During the last beet campaign 2003 further full scale trials were carried out in other factories of *Agrana*. Due to goals of energy saving and high beet slicing capacity, the factories used myristic acid very carefully, in order to keep a certain lactic acid level with a positive influence on pulp pressing. At *Leopoldsdorf* the first additions of 10 g/t of beet to the mixer with 6 shocks per day

did not show any effect (Figure 14). A first positive result was achieved with 10 g/t to mid-tower, but the drop in lactic acid was slow and the dosing rate was obvious close to the limit. Therefore the rate was increased to 20 g/t of beet, with tolerable influence on pulp pressing. Lactic acid in raw juice dropped to 400 mg/L in the first trial and to 300 mg/L in the second trial. In addition to lactic acid, two parallel drops in glucose are visible, but the first one was caused by a coincidental change in glucose content of beet, according to additional analysis of pressed beet juice.

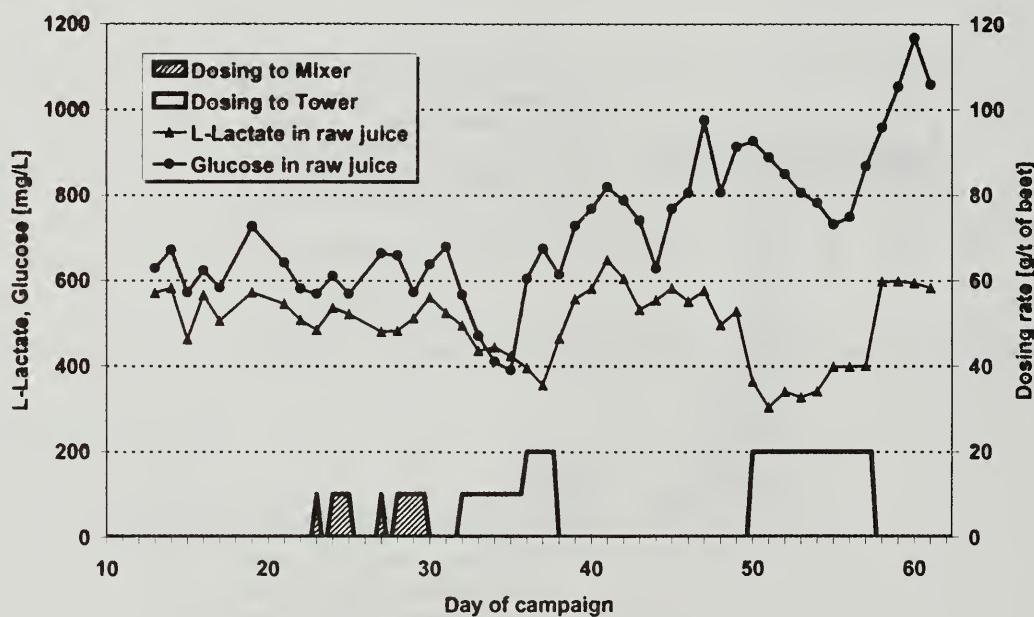


Figure 14. Full scale trials at Leopoldsdorf during the beet campaign 2003.

Further trials were carried out at *Hohenau*, but with a uniform dosing rate of 20 g/t of beet to the tower (position low-tower and later position mid-tower), with 6 shocks per day (Figure 15). Lactic acid levels dropped from 700 mg/L and above down to 400 mg/L, and two parallel drops in glucose are visible, which were - in contrast to *Leopoldsdorf* - not caused by a coincidental change in beet quality, according to pressed juice analyses. Unlike lactic acid, glucose increased during the course of the campaign and it was necessary to plot it with the doubled scale on the right. At the end of the campaign, when the stock of myristic acid was empty, rosin acids were used to lower the levels of lactic acid and glucose. As all daily figures were analysed by the *Yellow Springs* equipment directly in the sugar factories, we don't have parallel fructose results.

Residue Studies

In combination with full scale trials, residue studies have been started. The fatty acids are insoluble in water and only their alkali soaps are water soluble, but precipitate with calcium. In connection with hardness of water, everybody knows the behaviour of soaps. Chemists may know that potassium palmitate is a titrant for calcium, due to the low solubility of calcium palmitate. For myristic acid and lauric acid a higher solubility is expected, but in the same order. Thus a high removal during juice purification was expected. In Table 6 first residue results for collected samples are shown, taken from trials with a dosing rate of 20 g myristic acid per ton of beet (6 shocks per day), as well as some results for free myristic acid in well-known products.

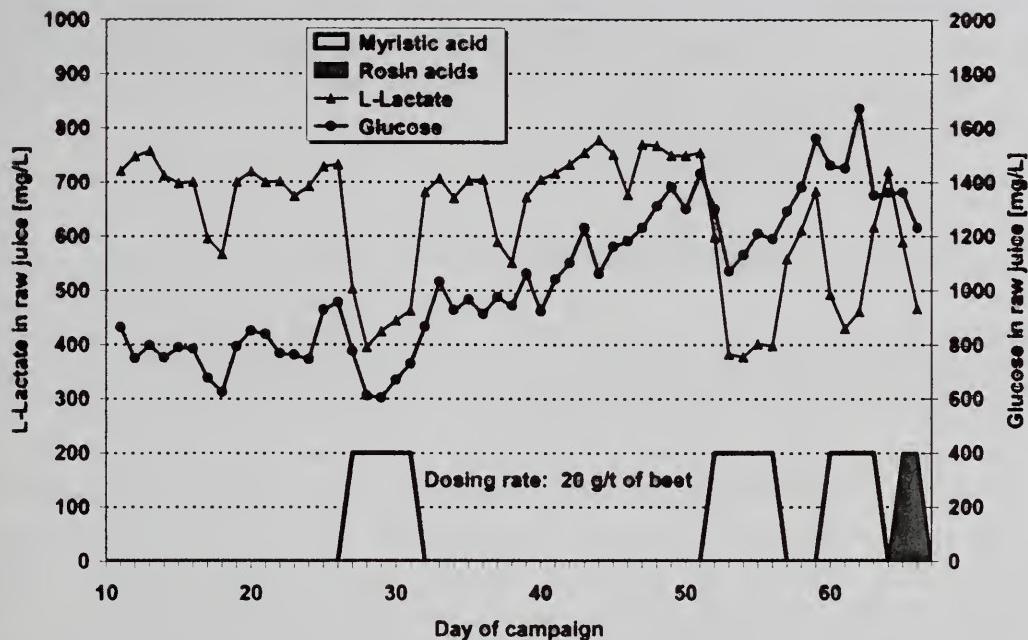


Figure 15. Full scale trials at Hohenau during the beet campaign 2003.

Table 6. Myristic acid residues (mg/kg) after dosing of 20g/t of beet.

SAMPLE	Dosing to mixer	Dosing to mid-tower
Raw juice	10.5	3.8
Pressed pulp, 30 % D.M.	12.3	34
Dried pulp, 90 % D. M.	9.5	10.7
Lime sludge, 65 % D.M.	4.8	2.5
Thin juice	0.79	0.85
Thick juice	1.2	1.7
Molasses	7.4	3.8
White sugar	0,21	0.12
Coconut oil (free acid)		30
Butter (free acid)		100
Lip balm (free acid)		17 000

Depending on the dosing point, myristic acid moves partly with the cossettes and there is a loss during pulp drying. Precipitation with lime caused a loss of concentration during juice purification, as expected. Obviously there is some further loss with steam during evaporation, as the values from thin to thick juice, based on product, show only a twofold enrichment instead of a fourfold one. Some results of the interim products cannot be explained logically, e.g. the results for thick juice, compared to raw juice and the mode of dosing. Side effects could lead to

unexplainable results, but are unknown up to now. The residues in molasses are similar to those of hop beta acids and rosin acids and the traces in white sugar are low. For comparison, coconut-oil, butter and lip balm were added as well-known examples from everyday life. These products show a considerable content of free myristic acid. In addition to the free acid, these fat products consist of up to 10 % myristic acid, bound to glycerol.

DISCUSSION

A third natural antibacterial aid from plants - myristic acid - is presented to the sugar industry. Several laboratory experiments, such as slide culture method, bacterial staining, drop in optical density and release of invertase, confirm a disintegration of bacterial cells after myristic acid addition. References of the present paper point to a disintegration or lysis of cells by fatty acids and are in agreement with the present findings. As a drop in optical density after product addition had been found for hop beta acids and rosin acids in the past [12], it is concluded that disintegration of Gram-positive bacteria is common for all these water insoluble acids. Additionally bacteriostatic effects could arise, which were referred to as "bacteriostatic power" of hop acids in old textbooks of the brewing industry [42]. Because of different mechanisms of effect, the term "minimum inhibitory concentration" (MIC) was replaced by "minimum effective concentration" (MEC) in this paper, in order to comprise all variants, from growth inhibition to cell disintegration.

Cell disintegration, caused by myristic acid, was used to demonstrate the existence of both, bacterial endo-invertase and bacterial exo-invertase. The activity of endo-invertase is not detectable without disintegration of cells. The effect of exo-invertase, produced by growing bacterial cells, is visible from full scale trials, which show a parallel drop in glucose and lactic acid after dosing of antibacterials. Invertase from beet is not affected by these natural products and so any monosaccharide increase from pressed beet juice to raw juice, caused by beet invertase, will not correlate to dosing of natural antibacterials.

No effect was achieved with myristic acid at temperatures of 35°C and 45°C, because insensitive Gram-negative bacteria are able to grow at these temperatures. Therefore fatty acids cannot be classified as disinfectants. Positive reports with pure strains created some enthusiasm in the past, but in the long run a second selective criterion, such as high temperature, is necessary to achieve a permanent effect. Temperatures used in beet extraction and cane "diffusion" are advantageous, compared to cane mills with low temperature operation. Furthermore, no effect can be expected with myristic acid in slight alkaline juices, such as thin juice, thick juice or in alkaline beet extraction, because of the considerable increase of the minimum effective concentration at neutral or slight alkaline pH values.

In spite of the selective mechanism of high temperatures, adaptation of bacterial strains is possible after some time of application. It is difficult to demonstrate adaptation in the laboratory scale and again difficult to schedule adaptation in full scale. There was one opportunity to re-establish an effect of rosin acids by myristic acid in the beet campaign 2002. Although all natural compounds are water insoluble acids with similar effect on bacterial membranes, the relationship is obviously low enough to displace strains which are insensitive against one of the natural acids.

A natural antibacterial should not be changed during a period with good effect, but only in case of diminishing effect. It is difficult to plan a change of the natural products, but alternatives should be at hand to displace insensitive strains if necessary.

CONCLUSION

- After hop beta-acids and rosin acids, myristic acid has been found as a third natural antibacterial aid for the sugar industry.
- A precondition for getting an effect is the selection of Gram-positive thermophilic bacteria by high temperatures, usual in beet extraction and cane „diffusion“.
- Compared to hop beta acids, higher amounts of effective substance are necessary and less effect will be reached at neutral or slight alkaline pH.
- Long-chain fatty acids from palm kernel oil with chain lengths from C12 to C16 are of importance, with myristic acid C14 as the favorite one. Myristic acid is considered harmless and is a well-defined product. The glycerol ester and traces of free acid are normally consumed with butter and coconut oil.
- Kosher certificates will be necessary to confirm the origin from plants.
- In the sugar industry an aqueous soft soap solution could be used.
- To overcome adaptation of strains even at high temperatures, alternation with hop beta acids and rosin acids is necessary.
- The right combination of natural products for an individual factory has to be found and, as the saying goes: “Never change a winning team”.

APPENDIX

Details on materials and methods

- Deep-frozen raw juice samples from the *Agrana*-factory *Tulln* (20 mL) have been used as inoculum for trials with impure laboratory cultures (500 mL).
- Type strains of *Bacillus stearothermophilus*, now *Geobacillus stearothermophilus* (ATCC 12980 = DSM 22) and *Clostridium thermohydrosulfuricum*, now *Thermoanaerobacter thermohydrosulfuricus* (ATCC35045 = DSM 567) have been ordered from the German type culture collection (DSMZ) [43].
- Strain *Geobacillus stearothermophilus H5-65*, isolated by Hollaus [39], was kindly supplied by the *Center for Ultrastructure Research* (now: *Center for Nanobiotechnology*) at the *University of Natural Resources and Applied Life Sciences (BOKU)*, Vienna, Austria.

- For aerobic micro-organisms a medium was used, close to “medium VIII” of *Bartelmus* and *Perschak* [37], but without agar. Composition per litre: Bacto-peptone 10 g, yeast extract 5 g, meat extract 5 g, K₂HPO₄*3H₂O 1.3 g, MgSO₄*7 H₂O 0.1 g, FeSO₄*7H₂O 0.02 g. Sucrose was added according to individual trials: a) 5 g/L to add up the sucrose content from raw-juice inoculum and b) 10 g/L, added as 40 % sterile-filtered solution, for pure strain trials.
- For *Thermoanaerobacter thermohydrosulfuricus* a medium was used, close to medium 61 of DSMZ [43]. Composition per litre: Sucrose 10 g, tryptone 10 g, yeast extract 2 g, FeSO₄*7H₂O 0,2 g, Na₂SO₃ 0.2 g, Na₂S₂O₃*5 H₂O 0.08 g. To avoid introduction of oxygen, sucrose was added before autoclaving. For pre-cultivation of anaerobic strains a semi-solid medium with addition of 3 g agar per litre was used.
- Palm kernel fatty acids were used as technical products with 98% purity, either dissolved in ethanol (1 %) or as aqueous potassium soap solution. Other fatty acids or related compounds were used as pure chemicals and all dissolved in ethanol (1 g/100 mL). The solvent ethanol had no effect on the tested cultures, even at the highest product concentrations under test (150 mg/L).
- Laboratory trials were carried out in sterile glass vessels (500 mL) with pH recording and slow magnetic stirring, in order to avoid artificial aeration and to simulate conditions in beet extraction towers. With the lowest intensity of magnetic stirring after inoculation with semi-solid medium from *Burry* tubes, and with normal stirring after growing on, this method even proved sufficient for *Thermoanaerobacter thermohydrosulfuricus*, without N₂ or an anaerobic chamber.
- In addition to the most important information from pH-recording, samples were drawn for measurement of optical density, microscopy, glucose, fructose and lactic acid determination.
- Optical density was measured against air at 600 nm and reported as difference against substrate.
- A slide culture method was used as described by *Bartelmus* and *Perschak* [37], but with 10 µL of culture plus 0,4 - 0,5 mL of medium “VIII”, spread over 10 cm² of area on the slide (4 cm x 2,5 cm).
- Bacterial cells were stained by the Congo red/blue method [38], but with 1 cm² film area instead of 10 cm², as reported in the original paper.
- For laboratory trials, glucose and fructose were analysed by the enzymatic hexokinase method, and L- and D-Lactate by another enzymatic method. Raw juice samples from full scale trials were analysed for L-lactate and glucose by a *Yellow Springs* equipment.
- Fatty acid residues were determined by ion-suppression chromatography, as a sampler was available for this method. Column: IonPac NS1, particle size 10 µm, with appropriate guard column. Eluent: Acetonitril+methanol+HCl 0,05mM (60v+24v+16v). Flow rate: 1 mL/min at 42°C. Detection: suppressed conductivity. Suppressor: AMMS ICE II with 2,5 mM KOH solution as regenerant.

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SUCCESSFUL APPLICATION POINTS TO CONTROL BACTERIAL INFECTIONS THROUGHOUT SUGAR FACTORIES USING BETA ACIDS/BETASTAB®10A

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ABSTRACT

Over the past 5 years BetaStab®10A has emerged as an exceptional natural biocide being applied to the extraction stage of sugar processing to successfully control bacterial infections. More recently studies have shown that the efficacy of BetaStab®10A is evident over a wide range of conditions and variables, including temperature and pH. Furthermore, due to its natural properties, it can be added at later stages of sugar processing without any risks of toxic residues remaining in the final product. As a result, BetaStab®10A is successful at combating infection in many additional stages and diverse environments that are found in sugar factories, examples of which are discussed within this presentation.

INTRODUCTION

Over the past seven years, the use of hops to control bacteria during sugar beet extraction has been demonstrated in many factories world-wide (Pollach *et al.*, 1999; Fowers, 2001). More recently the success of using BetaStab®10A to control bacteria throughout the whole sugar process has been recognised. Unlike some biocides which break down at high temperatures or are inactive over certain pH or temperature ranges, BetaStab®10A has been shown to be effective at a wide range of temperature and pH levels found at different stages of sugar processing. Additionally the safe, non-toxic nature of this water-based solution enables safe and easy dosing in any part of the factory without the need for expensive dosing systems or strict exposure control.

MATERIALS AND METHODS

Bacterial strains: *Leuconostoc mesenteroides* subsp. *dextranicum* strain (DSM20187) and *Bacillus stearothermophilus* (DSM22) were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) GmbH culture collection, Germany.

Culture media: Cultures of *L. mesenteroides* were incubated at 25°C under aeration in Man Rogosa Sharpe medium (MRS) (Merck). For pH studies, pH of MRS was altered with NaOH. Cultures of *B. stearothermophilus* were incubated at 60°C under aeration in Brain Heart Infusion medium (Oxoid). For studies into the growth of nitrite-producing bacteria, cultures were incubated at 60°C under aeration in 0.5% Bacto-Tryptone (Becton, Dickinson and Company), 0.25% Bacto-Yeast (Becton, Dickinson and Company), 0.01% NaNO₃ (Sigma), 2.6% Bacto-Agar (Becton, Dickinson and Company) and 0.1% Glucose, pH was altered to between 8.6-9.1 using NaOH (Nitrite media). For studies into the growth of bacteria from dust boxes, cultures were incubated at 25°C under aeration in Plate Count Agar (PCA) (Merck).

Determination of minimum inhibitory concentration (MIC): The MIC was determined using the broth micro dilution assay. In brief, an overnight culture was diluted appropriately to obtain OD₆₀₀ = 1.0 (8 x 10⁸ organisms). 30µl of this was added to 3ml fresh broth (giving a final concentration of approximately 8 x 10⁶ organisms) and incubated appropriately with controls containing no BetaStab®10A, but the same volume of 10% ethanol solution (that which was used to make BetaStab®10A dilutions). Following incubation, growth quantification was achieved by measuring optical density at 600nm.

Determination of bacterial susceptibility to beta acids in factory samples: These studies were conducted using the agar and/or broth micro dilution assay. For nitrite studies, nitrite media was inoculated with 100µl thin juice and incubated for 36h at 60°C under aeration. Either 2ppm, 10ppm or 20ppm beta acids were incorporated into the agar or broth and a negative control, containing no beta acids, was included. Bacterial growth was assessed visually on a scale of zero (no growth) to four (growth of control) for the agar assay and nitrite levels in the broth assays were measured by nitrite-test strips (Merck). For the dust box studies, the agar dilution method was used as described above but using PCA agar incubated at 25°C.

RESULTS AND DISCUSSION

1. BEET EXTRACTORS AND CANE MILLS AND DIFFUSERS

It has been demonstrated using broth micro dilution assays to obtain MIC values for pure bacterial cultures, that BetaStab®10A is active over a wide temperature range as represented by the figures below. These experimental culture conditions mimic those found at factory application points that may operate at high or low temperatures.

These charts (Figure 1a/1b, shown below) show the growth of *Leuconostoc mesenteroides* subsp. *dextranicum* at 25°C and *Bacillus stearothermophilus* at 60°C in the presence of the various

concentrations of beta acids. Each data point represents the mean from triplicate samples, and the error bars show the standard deviation.

Figure 1a

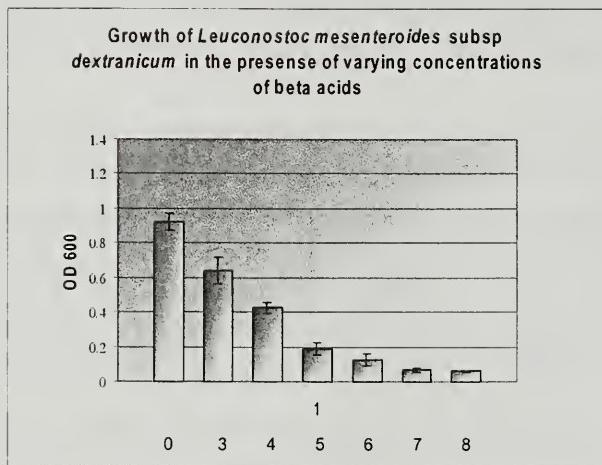
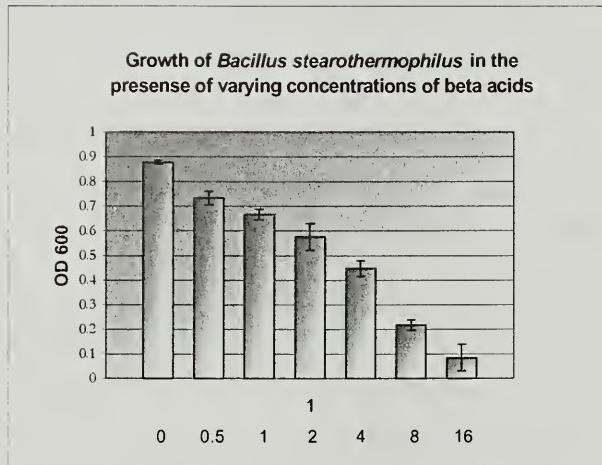


Figure 1b



These results demonstrate that beta acids are effective at inhibiting the growth of *Leuconostoc mesenteroides* subsp. *dextranicum* and *Bacillus stearothermophilus* and thus demonstrate the efficacy of beta acids over a wide temperature range, from 25 - 60°C.

2. HEAT EXCHANGERS

Laboratory and factory trials have shown that BetaStab®10A retains its activity despite heat treatment at 90°C 15 minutes, 100°C 15 minutes or 110°C 15 minutes. The minimum inhibitory concentration for beta acids against *L. mesenteroides* remained at 8ppm (data not shown) following the above heat treatments demonstrating that beta acids in factories are not required to be re-administered following passage through extensive heat.

3. PRE-LIMING

It has been demonstrated in laboratory experiments using pure bacterial cultures that BetaStab®10A is active over a wide pH range as represented by the figures below. These experiments mimic conditions found at factory application points that operate at extremes of pH.

These charts (Figure 2a-c) show the growth of *Leuconostoc mesenteroides* subsp. *dextranicum* grown at optimal temperatures in the presence of varying concentrations of beta acids over a wide pH range. Each data point represents the mean from triplicate samples, and the error bars show the standard deviation.

Figure 2a

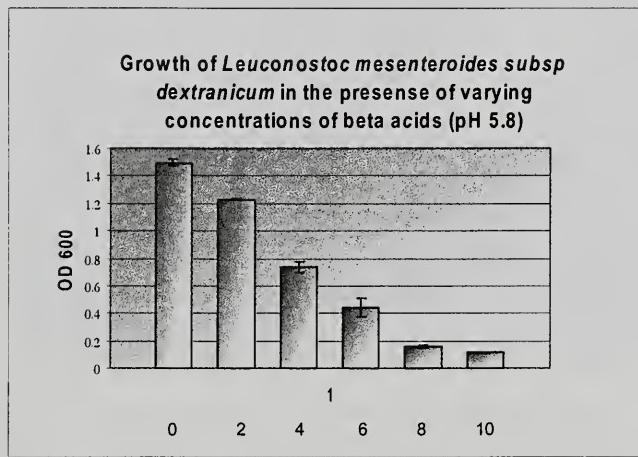


Figure 2b

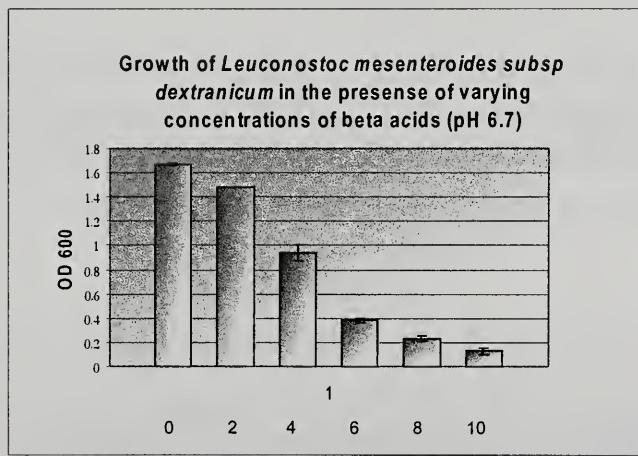
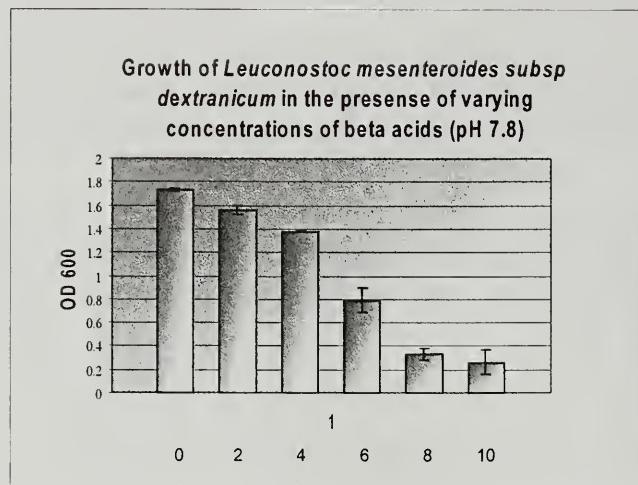


Figure 2c



These results demonstrate that beta acids are effective at inhibiting the growth of *Leuconostoc mesenteroides* subsp. *dextranicum* over a wide pH range with only subtle differences in the minimum inhibitory concentration between the pHs tested. In all cases 8ppm beta acids reduced growth with respect to the control more than 80% irrespective of the pH of the media. The alkaline range of the experiments did not exceed 7.8, as over pH 8.0 the growth of *L. mesenteroides* was severely impaired.

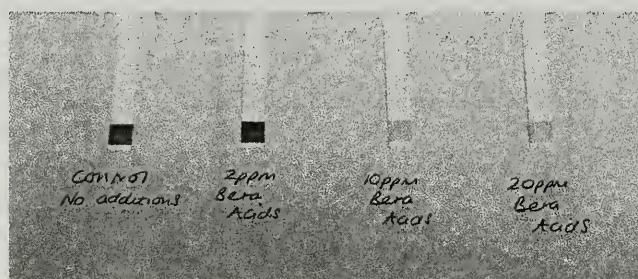
In support of these laboratory experiments it has also been shown in recent trials conducted by American Crystal (Samaraweera *et al.*, 2004) at their Drayton factory that administration of BetaStab®10A in their pre-limer resulted in a clear reduction in nitrite levels and a 1 log unit decrease in *Leuconostoc* counts.

4. THIN JUICE/ SYRUP

During a recent factory trial conducted by BetaTec, samples of thin juice were taken when there was a high nitrite level (80ppm); an indirect indicator of bacterial infection. Laboratory experiments indicated that 10ppm beta acids could reduce nitrite levels from 80ppm to 2ppm as shown in Figure 3a.

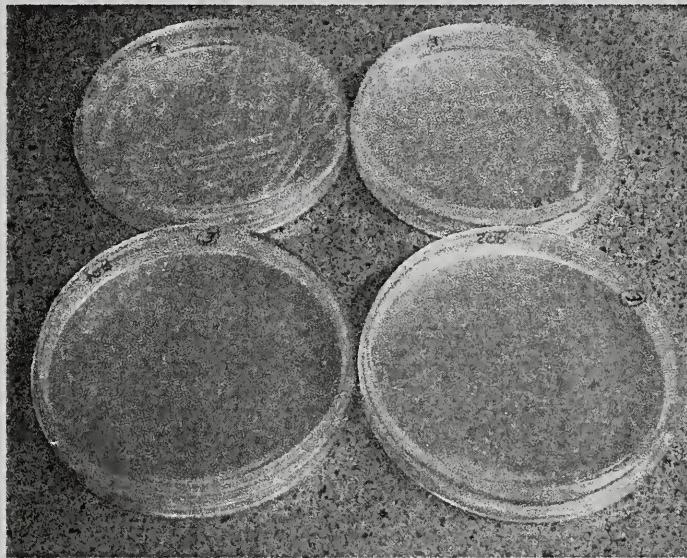
Figure 3a

Results of Merck Nitrite-test strip tests conducted on thin juice mixed culture



The decrease in nitrite levels was demonstrated to be due to the inhibition of at least 4 strains of nitrite-producing bacteria isolated from the thin juice that were inhibited by 10ppm beta acids in agar dilution tests, see Figure 3b.

Figure 3b. Using one of the colony types to exemplify all, this demonstrates that these nitrite-producers isolated as pure bacterial cultures were inhibited/killed by 10ppm beta acids



From top left clockwise;
control no biocides; 2ppm beta acids;
10ppm beta acids; 20ppm beta acids

5. THICK JUICE

Recent pilot scale trials into the use of beta acids to protect thick juice during storage (Hein *et al.*, 2002) have demonstrated that beta acids can delay a pH fall and invert sugar production. As beta acids are not active against yeasts and molds, additional protection may be required on the surface, such as NaOH.

6. DUST BOXES

During a recent factory trial conducted by BetaTec, samples from a dust box were taken and analysed. Growth in the presence of 20ppm beta acids was found to be reduced up to 50%* when compared to growth of a negative control, suggesting that beta acids may be useful in controlling bacterial growth at these points.

*The reduction observed exhibited variability between factories (this variability was attributed to differences in the bacterial species present in each independent factory).

7. SUMPS/DRAINS

Beta acids can also be added in the sumps and drains of sugar factories, to limit any inoculation of bacteria when these areas are added back into the process.

CONCLUSION

Laboratory trials, pilot-scale studies and factory trials have all successfully demonstrated the versatility of beta acids as an antibacterial agent. A critical attribute is that the activity is unaffected by extremes of temperature and pH. This, together with its non-toxic nature, make it an ideal agent to control infection at any point in sugar processing and eliminates the requirement to use multiple antibacterials and re-administer after hostile conditions.

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THE IMPACT OF USING RAPID ACTION BIOCIDES FOR MILL AND CANE SANITATION ON QUALITY OF SUGAR AND MOLASSES

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ABSTRACT

Sugarcane juice is very nutritive and can inhibit the action of many biocides. The time required to kill microbes is also an important factor for achieving optimum economic benefits. During milling, only 15 to 20 minutes are available for biocide to kill microbes. The fiberizer used for better cane preparation acts as a source of further microbial infection of the cane, and it is not usually treated in mill sanitation programs. For treatment of the fiberizer, the biocide must act within a few minutes. When biocides capable of killing a significant portion of mesophilic as well as microbes capable of growing at higher temperature (about 90⁰C) in 1 minute for cane sanitation and 10 minutes for mill sanitation are used, there should be a minimal rise in reducing sugar as well as acidity during the entire process. The improved quality will be reflected in final molasses and sugar.

Biocides not yielding such benefits are not economical. Quaternary ammonium compounds (Quats) and dithiocarbamates (DTC) are currently used for mill sanitation according to US FDA guidelines. Quats are inhibited by calcium, organic matter and bagasse, and may not kill microbes effectively in cane juice; DTCs require more than 25 minutes in cane juice at 10 ppm dose to kill about 90% microbes. Formulations of synergistically acting dithiocarbamates with permitted activators and penetrating agents can be made to achieve about 90% killing of microbes in 1 minute and in 10 minutes (Indian Patents application no. 306 / BOM / 99).

The biocide formulations are thus ideal for cane sanitation (**Biocide 01**) or for diffuser sanitation and mill sanitation (**Biocide 10**). Experiments were conducted in a sugar factory to evaluate various biocides for one week each. A minimum of 4 two-hourly composite samples of primary juice, mixed juice, clear juice, syrup and final molasses were collected (mercuric chloride used

as preservative). Reducing sugars and acidity by titration were determined. Data was collected for a week using 10 ppm Quats, 10 ppm DTC mixture, 10 ppm **Biocide 10** and 5 ppm **Biocide 01** prior to the fiberizer with 10 ppm **Biocide 10** for mill sanitation. Samples of sugar per shift were also collected. Sugar samples and final molasses samples were analyzed by HPLC for polysaccharide, glucose, fructose and sucrose. Results showed that **Biocide 10** was effective in reducing microbial sugar losses to molasses and reduced polysaccharide in molasses and sugar. Cane sanitation using **Biocide 01** reduced microbial losses to minimal levels and significantly reduced polysaccharides in sugar.

INTRODUCTION

The use of biocides is seldom considered beneficial. Rather, biocides are considered a luxury. Hitherto, many chemicals have been tried for mill sanitation. These include oxidizing chemicals such as chlorine, iodine, hydrogen peroxide, peroxyacetic acid, ozone and chlorine dioxide, as well as non-oxidizing ones like quaternary ammonium compounds and dithiocarbamates. However, the economics of such use has never been demonstrated beyond doubt. In fact, Australian sugar mills do not use any biocides.

The reasons for this confusion in regard to the use of biocides for mill sanitation could be:

1. Micro-organisms are microscopic and cannot be seen with the naked eye, hence there is either ignorance or too much fear about their existence.
2. Strong (and wrong) belief that the boiling of juice kills all microbes and there is no microbial sugar loss after juice heating.
3. Biocides are evaluated by judging “purity drop” from PJ to MJ, a method much criticized and known to be incorrect.
4. Other methods of evaluation of PJ and MJ may not be reliable due to possible errors in sampling.
5. Nutrients, organic matter, reducing matter, bagasse, mud and dissolved solids can inhibit biocidal action of many chemicals, such as oxidizing biocides and quaternary ammonium compounds.
6. The time available to the biocide to kill the microbes in case of milling tandems is about 20 minutes and that for diffuser mills is less than 5 minutes.

The last reason is perhaps the most important and crucial as far as the efficiency and economics of the use of biocides for mill sanitation is concerned. Microbiological activity changes after 20 minutes for milling and 5 minutes for diffuser. Micro-organisms capable of growing at high temperature, which remain dormant, find the environment suitable for growth and the mesophilic microbes enter the dormant phase—or die—due to the thermal shock. “Safety” is one of the most important criteria, and an obligate requirement, for choosing a biocide for mill sanitation. The biocide must not remain in the sugar. Thermal instability of simple low molecular weight dithiocarbamates and their quick degradation, above 80 degrees C, to non-toxic products makes them a “safe” biocide for mill sanitation. However, this does not make them an *effective* biocide,

as the time required to kill the microbes in cane juice is more than 25 minutes. Hence, the situation worsens and the confusion persists because, theoretically, none of the chemicals has the potential to kill a majority of the microbes in cane juice within the available time, with the result that the use of biocides does not give any significant economic advantages.

Working on the hypothesis that using a formulation instead of a single molecule would result in better efficiency, the killing time required for various dithiocarbamates was studied. Sodium N dimethyl dithiocarbamate (40% aq), the most widely used biocide for mill sanitation in India, requires 45 minutes to kill about 90% microbes in cane juice when used at 10 ppm concentration. Its combinations with ethyl and cyano-imido dithiocarbamates can kill microbes in 25 minutes (same dose, same concentration). Taking advantage of such synergistic action of different dithiocarbamate molecules with the addition of certain inert penetrating and activating agents, we have prepared two formulations of biocides, namely, **Biocide 10** capable of killing about 90% microbes (including dormant thermophilic bacteria) at 10 ppm dose in *10 minutes* and **Biocide 01** capable of killing microbes in just *one minute*.

Apart from the time factor, another critical factor is a proper and reliable method of evaluation. There are only two stable products in any sugar factory, viz. sugar and molasses. As they are produced after the juice is concentrated manifold, they can reliably represent a large quantity of sugarcane. Further, their stability with respect to time can make them a reliable sample. Being the only final products of the process, any changes made in the process should be visible in any analysis of sugar and molasses. When we think of evaluating the efficiency of chemicals in eliminating/reducing the growth of micro-organisms during the process, we should be able to observe a reduction in their metabolic products throughout the process as well as in the two final products. Microbial growth will produce inversion products (reducing sugars), acids, oligosaccharides, and polysaccharides such as dextran and levan. The killing of microbes should reduce all these metabolites throughout the process and should be reflected in the final quality of the sugar and molasses with respect to the presence of these metabolites.

MATERIALS AND METHODS

The evaluation of these biocides was conducted in a 3500 TCD factory for the period of one week using benzalkonium chloride 10 ppm dose; dithiocarbamate mixture (40%) 10 ppm dose for 5 days; rapid action **Biocide 10** dose 10 ppm on mills for 10 days and **Biocide 01** spray on prepared cane before fiberizor 5 ppm dose and 10 ppm **Biocide 10** on mills for 5 days.

During the entire period, four two-hourly composite samples of primary juice, mixed juice, clear juice, unsulfured syrup and final molasses were analysed daily for reducing sugars by the Lane & Eynon method, acidity by titration with 0.1 N NaOH (pH 8.3 as end point) and brix by hydrometer / hand refractometer. Molasses and sugar samples were also analysed using calcium nitrate column, mobile phase water 0.5 ml per minute, column temperature 80° C, Lachrom L7110 pumps and Lachrom L 7490 RI detector (Merck – Hitachi).

RESULTS AND DISCUSSION

During the use of benzalkonium chloride for mill sanitation, reducing sugars (RS) in mixed juice (MJ) were found to be lower than those in primary juice (PJ) in about 42% of the samples analysed. This could be due to the higher microbial activity that degrades reducing sugars to various metabolites including acids and dextran. The higher rise in acidity by titration from PJ to MJ confirms more microbial activity. This degradation of RS dropped to about 20% when DTC mixture was used, whereas there was no evidence of RS destruction when **Biocide 10** and **Biocide 01** were used. The readings of this destruction were deleted while taking the average values for calculating inversion losses. The average values of the rise in RS and acidity during the entire experiment are noted in Table 1. Rise in RS from PJ to MJ was noticed to be about 42.3% when BKC was used, which reduced to about 29.6% with DTC mixture; 13.4% in case of **Biocide 10** and 13% when **Biocide 01** was used for cane sanitation with **Biocide 10**. The rise in acidity from PJ to MJ was found to be 27.5%, 17.4%, 6.29% and 5.3% respectively. This impact continued and the final molasses had the lowest acidity and RS during the use of **Biocide 01** and **Biocide 10**.

Biocide 01 is aimed at reducing sugar losses from prepared cane to 1st mill in addition to other benefits. It is noteworthy that there is significant reduction in RS and acidity, 29% and 18.5% respectively, of primary juice. Acidity of clear juice is also the lowest during this treatment, which denotes proper control over thermophilic bacteria. Fluctuations in the rise in acidity and RS can also be considered as one of the parameters to monitor microbial activity; lower fluctuations indicate lower microbial activity. Chart 1 is a plot of the rise in RS and Chart 2 is a plot of the rise in acidity from PJ to MJ. They clearly indicate the lowest fluctuations when **Biocide 10** and **Biocide 01** were used.

Samples of molasses and sugar were analyzed by HPLC / HPIC to determine polysaccharides, glucose, fructose and sucrose and the data are given in Table 2. Observations of analyses by titration are confirmed and there is significant reduction in the polysaccharide content of sugar and molasses due to the use of **Biocide 10** and **Biocide 01**, together with a reduction in the glucose, fructose and sucrose concentration in the final molasses. Reduction of sucrose in the molasses could be due to the indirect effect of the dextran reduction resulting in better molasses exhaustion.

Calculations of sugar loss: Loss of recoverable sugar per MT cane crushed was calculated using the average rise in RS and acidity with use of biocides by:

I } Using data of invert sugar: Sugar loss kg per MT cane crushed

$$= \text{Rise in invert sugars from PJ to MJ} \times \text{Av. brix PJ} \times 0.072 *$$

II } Using acidity data: Sugar loss kg per MT cane crushed =

$$\text{Rise in acidity from PJ to MJ} \times \text{Av. brix PJ} \times 0.0131 *$$

Total Sugar loss kg / MT is total of II & I

Data about sugar loss during use of various biocide treatments, given in Table 3, reveals that the use of **Biocide 10** and **Biocide 01** does have significant economic advantage.

Table 1. Reducing sugars and acidity by titration per 100 Brix

Biocide used	% Reducing sugars per 100 brix				Acidity by titration per 100 brix			
	PJ	MJ	Cl.J	FM	PJ	MJ	Cl.J	FM
Quat (BKC 50%) dose 10 ppm	1.84	2.60	2.55	10.64	10.74	13.42	7.43	24.79
DTC Mixture (40%) dose 10 ppm	2.30	2.98	2.61	11.13	10.84	12.76	5.82	25.96
Biocide 10 dose 10 ppm	2.16	2.46	2.54	10.58	10.01	10.64	4.78	19.81
Biocide 01 dose 5 ppm & Biocide 10 dose 10 ppm	1.53	1.72	1.82	10.45	8.67	9.13	4.36	16.79

Table 2. Analysis of final molasses and sugar by HPLC / HPIC.

Biocide used	% per 100 Brix final molasses				TPS ppm % in sugar
	TPS	Sucrose	Glucose	Fructose	
Quat (BKC)	16.06	44.79	6.11	7.94	185.55
DTC mixture	15.23	42.43	6.22	5.68	148.26
Biocide 10	14.46	40.20	5.98	5.30	99.69
Biocide 01 and Biocide 10	13.04	36.99	5.46	4.80	85.86

Table 3. Sugar loss kg / MT cane crushed and savings due to treatment.

Biocide used	Sugar loss kg per MT cane crushed			Sugar saving Kg / MT
	Inversion	Acidity	Total	
NO Biocide			2.52 assumed	-
Quat (BKC)	1.19	0.84	2.03	0.49
DTC Mix.	1.05	0.63	1.68	0.84
Biocide 10	0.44	0.18	0.62	1.90
Biocide 01 & Biocide 10	0.30	0.13	0.43	2.09
Additional gain in PJ	0.96	0.38	1.53	3.62

Chart 1. Rise in Reducing Sugar per 100 Brix from Primary Juice to Mixed juice

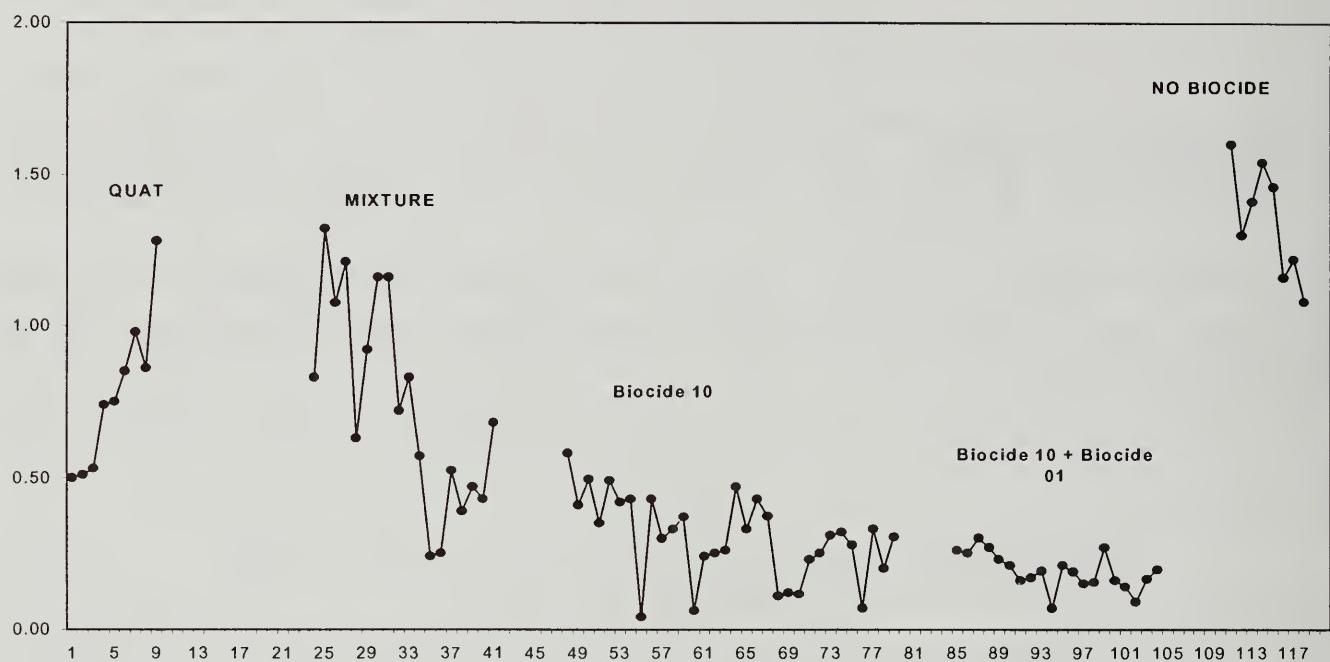
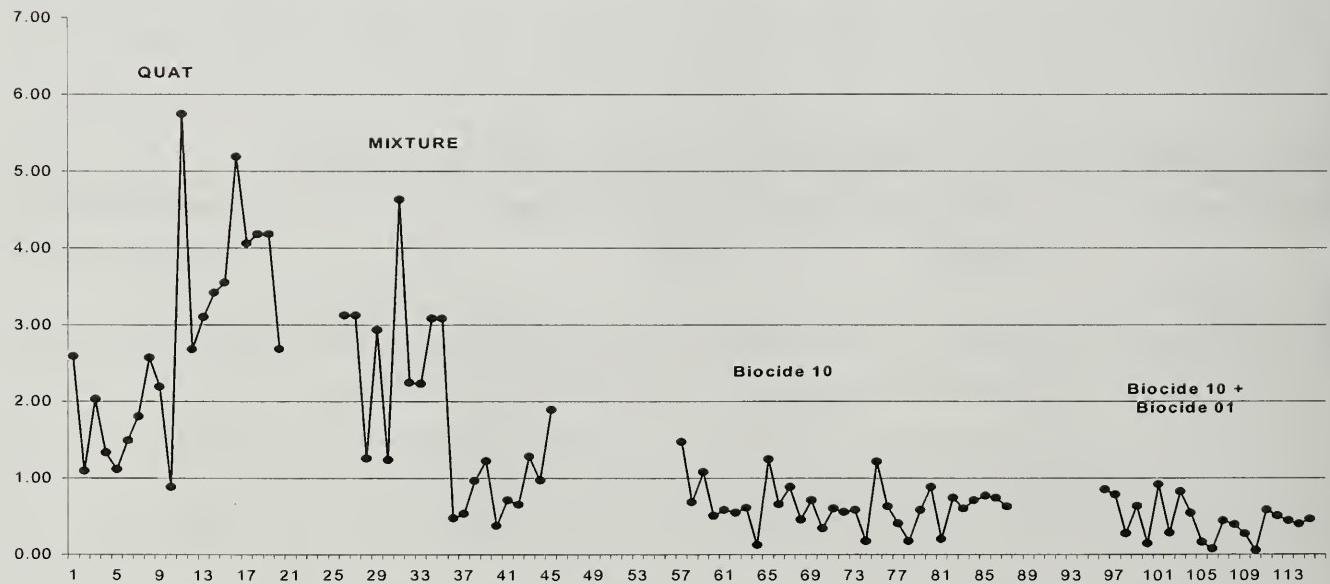


Chart 2. Rise in Acidity per 100 Brix from Primary Juice to Mixed Juice



INVESTIGATION ON THE EFFICIENCY OF CARBONATATION SLUDGE ADDITION INTO TURKISH FUELS FOR DESULFURIZATION OF FLUE GASES

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ABSTRACT

Nowadays, SO₂ emission is more strictly controlled due to the sensitivity toward environmental protection. As a result of this, use of either fuel containing low sulfur or reduction of SO₂ emission has gained importance. Reduction of SO₂ emission in flue gases by physical and chemical treatments is preferred by large industries in which a huge amount of fuel is used, since fuels containing low sulfur are very expensive.

There are many methods for desulfurization of flue gases in the world. One of the popular methods is the treatment of SO₂ with alkaline earth oxides, producing alkaline earth sulfates. The addition of carbonatation sludge into fuel provides not only the reduction of SO₂ emission, but also utilization of a sugar factory's by-product.

In this study, a laboratory method was proposed to determine the efficiency of carbonatation sludge addition into high sulfur containing fuel oil or coal used in Turkish Sugar Factories in order to determine the parameters needed for industrial applications.

INTRODUCTION

Control of SO₂ emission has been important due to the strict environmental rules. Therefore the industries have to be used either expensive fuels containing low sulfur or desulfurization system together with cheaper fuels containing high sulfur.

Large industries have preferred the use of cheaper fuels containing high sulfur together with an “SO₂ reduction system” instead of using expensive fuels. Some additives have been used in boiler systems before, during and after the combustion for desulfurization of flue gasses (ERC Combustion Tech., 2002).

Addition of alkaline earth metal oxides to fuel before combustion is the most popular and the most suitable technique to reduce SO₂ emissions in sugar industry. There are some applications of carbonatation sludge addition as a source of CaO into fuel oil or coal to reduce SO₂ emission in the sugar industry (Dolignier & Martin, 1997; Evertz, 1991). The importance of Ca/S ratio has been emphasized in the efficiency of desulfurization in the previous studies, but it has never been mentioned about the importance of kind of fuel itself.

In this study, a test method was proposed to determine the efficiency of carbonatation sludge addition into fuels and to determine the Ca/S ratio needed for a specific type of coal or fuel oil before an industrial application.

MATERIALS AND METHODS

Fuel oil no 6 produced in Turkey as a liquid fuel sample and coals from different origins (Alpullu, Kayseri, Bor) containing medium and high sulfur as fuel samples were used in this study. Carbonatation sludge from Ankara Sugar Factory was used as the CaO resource.

Coal samples were crushed and pulverized after air drying and passed through a 250 µ sieve in order to be prepared for the analyses. Carbonatation sludge sample was also prepared by passing through a 250 µ sieve after air drying. The same carbonatation sludge sample was used throughout the study.

Parameters used in this study were determined according to the following methods:

Coal

Gross calorific value – ASTM D 5865

Sulfur, total – ASTM D 3177

Sulfur, forms (Sulfate, pyritic and organic sulfur)-ASTM D 2492

Ash – ASTM D 3174

Moisture - ASTM D 3173

Nitrogen – Calculated according to the method explained in the test procedure section

Fuel-oil no 6

Gross calorific value – ASTM D 240

Sulfur, total – ASTM D 2494

Nitrogen – Calculated according to the method explained in the test procedure section.

Test Procedure

Experiments were carried out in the following steps.

1. Moisture; ash; sulfate, pyritic, organic and total sulfur contents of five coal samples (Table 1) and total sulfur; nitrogen and gross calorific value of one fuel-oil sample were determined (Table 2) in the first step, according to the methods mentioned above.
2. Particle size distribution by Malvern Mastersizer 2000 (Table 3) and chemical composition of carbonatation sludge (Table 4) were determined.
3. Fuel samples and carbonatation sludge were mixed and the mixtures, in which Ca/S mole ratios were from 0 to 2.5 were burned in a calorimeter bomb (IKA C 4000). Amount of carbonatation sludge has to be added into fuel to realize Ca/S ratio as 1.0, 1.5, 2.0, 2.5 was calculated using the following formula:

$$m_s = K \times m_f \times \frac{(\% S)_f / 32,06}{(\% \text{CaCO}_3)_s / 100} = K \times m_f \times 3,119 \times \frac{(\% S)_f}{(\% \text{CaCO}_3)_s}$$

m_s = Mass of sludge has to be added into fuel, g

m_f = Mass of fuel, g

K = Ca/S ratio, mole/mole

(% S)_f = Total sulfur content of fuel, %

(CaCO₃)_s = CaCO₃ content of sludge, %

4. Fuel-sludge mixtures were burned in a calorimeter bomb and the following steps were applied.

The two basic reactions in the bomb were :



CaCO₃ in the sludge sample was transformed to CaO [1] in the bomb. Some of SO₂ produced as the result of fuel combustion was neutralized by CaO formed under bomb conditions and precipitated as CaSO₄. The remaining SO₂ was not neutralized by CaO, and it was transformed to H₂SO₄ together with water in the bomb. H₂SO₄ formed in the bomb was assumed to be the source of SO₂ corresponding to the emission in real combustion conditions. Therefore the acidity of the solution obtained by washing the inside of the bomb could be a useful tool for calculation of SO₂ emission.

Since both HNO_3 and H_2SO_4 are formed when fuel was burned in a calorimeter bomb, H_2SO_4 only is not responsible for the acidity of the solution. In order to take into account the acidity coming from HNO_3 , the nitrogen content of fuel responsible for HNO_3 formed in the bomb was calculated from the data obtained as a result of the combustion in the bomb without using carbonatation sludge.

Acidity coming from HNO_3 was assumed constant for the same fuel sample for both cases, with or without carbonatation sludge addition. The only factor that changes the acidity is the amount of SO_2 that was not neutralized by CaO in the bomb for both cases.

Nitrogen and sulfur content of carbonatation sludge were determined by burning it in the bomb. Since no acidity was observed in the solution obtained from washing of the bomb after the combustion of the sludge sample only, it was seen that neither HNO_3 nor H_2SO_4 formed in the bomb as a result of sludge combustion. It was assumed that the sludge sample did not cause any additional acidity in the bomb in the case of sludge-fuel mixture combustion in this method.

The calculation method followed in this study is explained below:

m_f = Mass of fuel, burned in the bomb, g

m_s = Mass of sludge, added into fuel in the bomb, g

S = Total sulfur in fuel, according to ASTM D 3177, %.

S_f = Final sulfur in fuel, corresponding to SO_2 emission in case sludge is added into fuel, %

N = Nitrogen in fuel, calculated from acidity of bomb (it will be explained), %

V_T = Total volume of Na_2CO_3 solution (0.03547 M), spent in neutralization titration of the bomb washings, mL

V_N = Volume of Na_2CO_3 solution, spent for only HNO_3 in the bomb washings, mL

V_{Sf} = Volume of Na_2CO_3 solution, spent for only H_2SO_4 in the bomb washings corresponding SO_2 emission, mL

According to ASTM D 5865 and ASTM D 240, H_2SO_4 and HNO_3 are formed as a result of fuel combustion in the bomb. Acids formed are neutralized by Na_2CO_3 solution after burning to take into account the heat of formation of HNO_3 and H_2SO_4 in bomb conditions (Appendix of ASTM D 5865 and corrections of ASTM D 240).

0.03547 M solution of Na_2CO_3 was used for neutralization in this study for solid fuels as it is in ASTM D 5865. The following reactions take place during the neutralization of HNO_3 and H_2SO_4 with Na_2CO_3 :



In neutralization reactions [3] and [4], some part of the Na_2CO_3 is spent for H_2SO_4 and the rest is spent for HNO_3 . If sludge is not added into the fuel, total sulfur in the fuel will form SO_2 first and

H_2SO_4 second in the bomb conditions. In case of carbonatation sludge addition, some part of SO_2 reacts with CaO forming CaSO_4 and the rest of it is transformed to H_2SO_4 and then neutralized by Na_2CO_3 during the titration. SO_2 remaining in the bomb without reacting with CaO would be responsible for SO_2 emmission in a real combustion situation. If it is assumed that HNO_3 produced in the bomb is constant for both cases, combustion with or without additives, H_2SO_4 corresponding SO_2 emmission can be calculated using the data obtained from only the neutralization titration as the following.

In reaction [4] volume of Na_2CO_3 spent (V_N) for HNO_3 as mL;

$$V_N = m_f(\text{g fuel}) \times \frac{N(\text{g nitrogen})}{100(\text{g fuel})} \times \frac{1(\text{mole N})}{14(\text{g N})} \times \frac{1(\text{mole Na}_2\text{CO}_3)}{2(\text{mole N})} \times \frac{1(\text{L Na}_2\text{CO}_3 \text{ soln})}{0.03547(\text{mole Na}_2\text{CO}_3)} \times \frac{10^3 \text{ mL}}{\text{L}}$$

$$V_N = 10.069 \times N \times m_f \quad \{1\}$$

$$V_{Sf} = V_T - V_N \quad \{2\}$$

If V_T is determined experimentally after combustion and V_N is calculated from formula {1}, V_{Sf} could be calculated as an indicator of SO_2 emission.

$$V_{Sf} = m_f(\text{g fuel}) \times \frac{S_f(\text{g sulfur})}{100(\text{g fuel})} \times \frac{1(\text{mole S})}{32.06(\text{g S})} \times \frac{1(\text{mole Na}_2\text{CO}_3)}{1 (\text{mole S})} \times \frac{1 (\text{L Na}_2\text{CO}_3 \text{ soln})}{0.03547 (\text{mole Na}_2\text{CO}_3)} \times \frac{10^3 \text{ mL}}{\text{L}}$$

$$V_{Sf} = 8.794 \times S_f \times m_f \quad \{3\}$$

After V_{Sf} was found, S_f causes SO_2 emission could be calculated from formula {3} or the following equation can be derived from formulas {1}, {2} and {3}.

$$S_f = \frac{V_{Sf}}{8.794 \times m_f} = \frac{V_T - V_N}{8.794 \times m_f} \quad S_f = \frac{V_T - (10.069 \times N \times m_f)}{8.794 \times m_f} \quad \{4\}$$

This means that sulfur based on SO_2 emission (S_f) can be calculated using only the amount of fuel burnt in the bomb (m_f) and the total volume of Na_2CO_3 (V_T) spent for neutralization titration, if nitrogen in fuel (N) transformed to HNO_3 in the bomb was known before.

Then the desulfurization percentage was calculated using total sulfur content of coal (S) determined by ASTM 5865 and final sulfur content (S_f) found by formula {4} as in the following:

$$\text{Desulfurization, \%} = \frac{S - S_f}{S} \times 100$$

5. Percentages of SO₂ reduction versus Ca/S ratios were calculated according to the method explained above and the results were shown in Table 5 and Figure 1.
6. Finally, effect of carbonatation sludge addition on calorific value of fuel was examined by two ways (6.1 and 6.2) and the results were shown in Table 6.
 - 6.1a- Initial gross calorific value of fuel only was determined (Q_{initial} or Q_{in}) by ASTM.
 - 6.1b- Initial gross calorific value of sludge only was determined (Q_{sludge} or Q_s) by ASTM using benzoic acid as combustion aid and it was found to be 339 cal/g .
 - 6.1c- Expected gross calorific values of fuel and sludge mixtures burned in the bomb were calculated from the initial calorific values imagining they were mixed physically (Q_{expected} or Q_{exp}).
 - 6.1d- Gross calorific values of fuel-sludge mixtures were determined (Q_{found} or Q_{fnd}) by ASTM.
 - 6.1e- The differences between Q_{exp} and Q_{fnd} were calculated.
 - 6.2a- Final gross calorific values of fuels were calculated (Q_{final} or Q_{fin}) imagining sludge as combustion aid by ASTM.
 - 6.2b- The differences between Q_{in} and Q_{fin} were calculated.

RESULTS AND DISCUSSION

As can be seen from Table 5 and Figure 1, desulfurization effectiveness of carbonatation sludge addition to fuel changed with the kind of the fuel even if the same Ca/S ratio was applied.

Sludge addition was more effective for some kinds of fuels such as "Alpullu Bükköy coal" than the others. When Table 1 and Figure 1 are examined together, it may be concluded that the removal of organic sulfur might be easier than the removal of pyritic sulfur by this method. It is a matter of future investigations.

This study has shown that the sulfur content of fuel can be reduced by 70-75% using carbonatation sludge. As can be seen from Table 6, expected calorific values are not so different from the calorific values found in the bomb in case of carbonatation sludge addition. This means that there may not be any additional loss in calorific value apart from the loss of calorific value of fuel due to mixing it with sludge which has a low calorific value (339 cal/g).

CONCLUSION

In this study, it was surprisingly seen that desulfurization efficiency cannot be specified by the Ca/S ratio only. Since desulfurization efficiency depends on the kind of fuel, it is important to investigate the efficiency of sludge addition into the specific kind of fuel before a factory application. The method proposed in this study will be a usefull tool for the determination of parameters needed for a factory application.

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Table 1. Results of Coal Analysis

Origin of sample	Moisture, %	Ash, %	Sulfate Sulfur, %	Pyritic Sulfur, %	Organic Sulfur, %	Total Sulfur, %	Total Nitrogen, %	Gross Calorific Value, cal/g
Alpullu-Bükköy	4.51	24.03	0.13	0.80	7.55	8.48	0.12	5394
Kayseri	9.48	27.18	0.42	1.24	0.86	2.52	0.29	4775
Bor-Mengen 1	1.82	25.49	0.15	1.84	7.16	9.15	0	5689
Bor-Mengen 2	2.48	22.68	0.17	1.66	7.75	9.58	0	5794
Bor-Mengen 3	2.55	20.41	0.12	2.09	7.61	9.82	0	5962

Table 2. Results of Fuel oil no 6 Analysis

Total sulfur, % = 3.81

Total nitrogen, % = 0.82

Gross calorific value, cal/g = 10,036

Table 3. Particle Size Distribution of Carbonatation Sludge

Size of 10 %, μ < 3.8

Size of 50 %, μ < 15.0

Size of 90 %, μ < 50.3

Average Particle Size, μ < 29.1

Table 4. Composition of Carbonatation Sludge

Moisture, % = 3.51

Organic matter, % = 1.55

Ignition loss, % = 48.93

SiO_2 , % = 1.60

R_2O_3 , % = 2.50

SO_3 , % = 0.90

CaO , % = 42.18

MgO , % = 2.23

CaCO_3 , % = 75.33

MgCO_3 , % = 4.66

Table 5. Desulfurization effect of carbonatation sludge addition into fuels in different Ca/S ratios

	Final Sulfur, %	Ca/S	Desulfurization ,%		Final Sulfur, %	Ca/S	Desulfurization ,%
“Alpullu-Bükköy” Coal	8.48	0.00	0.0	“Fuel-oil No 6”	3.81	0.00	0.0
	3.36	0.99	60.4		3.01	0.98	21.0
	3.78	1.06	55.4		2.91	1.01	23.5
	3.30	1.12	61.1		3.06	1.01	19.6
	3.13	1.23	63.1		2.54	1.46	33.5
	2.71	1.54	68.1		2.63	1.47	31.0
	2.96	1.55	65.1		2.47	1.48	35.2
	2.11	2.00	75.1		2.60	1.55	31.9
	2.20	2.07	74.0		2.19	1.96	42.4
	2.20	2.12	74.1		2.15	1.97	43.6
	2.28	2.50	73.2		2.31	2.06	39.5
	2.17	2.52	74.4		2.27	2.07	40.5
	2.02	2.55	76.1		2.55	2.19	33.0
					1.53	2.41	60.0
					1.57	2.43	58.7
					1.40	2.46	63.4
					2.03	2.47	46.9
“Kayseri” Coal	2.52	0.00	0.0	“Bor-Mengen1” Coal	9.15	0.00	0.0
	2.09	1.01	17.2		7.55	0.99	17.5
	2.21	1.05	12.2		7.73	1.07	15.6
	1.91	1.46	24.3		7.55	1.09	17.5
	1.88	1.50	25.4		6.91	1.49	24.4
	2.01	1.99	20.1		5.92	1.61	35.3
	2.12	2.00	16.0		5.10	1.97	44.2
	1.87	2.52	25.8		5.42	1.98	40.8
	1.68	2.64	33.5		4.70	2.47	48.6
					3.58	2.48	60.9
“Bor-Mengen2” Coal	9.58	0.00	0.0	“Bor-Mengen3” Coal	9.82	0.00	0.0
	5.76	1.00	39.9		8.56	0.98	12.8
	6.85	1.00	28.5		7.47	0.99	23.9
	5.83	1.49	39.2		7.45	1.46	24.1
	4.97	1.51	48.2		6.55	1.50	33.3
	2.84	1.99	70.4		6.63	1.50	32.5
	3.63	2.01	62.1		6.12	1.95	37.6
	2.96	2.49	69.1		5.75	1.95	41.5
	3.31	2.50	65.5		5.36	2.00	45.4
					3.47	2.44	64.7
					3.82	2.49	61.1
					4.14	2.50	57.8

Table 6. The Effect of Carbonatation Sludge Addition into Calorific Values of Fuels

	FUEL-SLUDGE MIXTURE						FUEL-SLUDGE MIXTURE						FUEL		
	Initial	FUEL		FUEL		Fuel Final	Initial	FUEL		FUEL		Expected	Found	Final	
		Qin	Ca/S	Qexp	Qfind	Qexp-Qfind		Qin	Qfin	Ca/S	Qexp	Qfind	Qexp-Qfind	Qfin	Qin-Qfin
	cal/g	cal/g	cal/g	cal/g	cal/g	cal/g		cal/g	cal/g	cal/g	cal/g	cal/g	cal/g	cal/g	cal/g
“Alpullu-Bükköy”	5394	0.99	3999	3950	48	5324	70								
Coal		1.06	3943	3935	8	5382	12	2.19	7542	7546	-4	10042	-6		
		1.12	3894	3879	15	5371	23	2.07	7643	7634	9	10024	12		
		1.23	3796	3776	20	5363	31	0.98	8728	8723	5	10030	6		
		1.54	3561	3513	48	5315	79	1.01	8700	8655	45	9984	52		
		1.55	3556	3534	22	5357	37	1.01	8703	8671	32	9999	37		
		2.00	3277	3248	29	5343	51	1.46	8212	8184	28	10001	35		
		2.07	3233	3240	-8	5408	-14	1.48	8190	8203	-13	10053	-17		
		2.12	3205	3159	47	5308	86	1.47	8205	8193	12	10021	15		
		2.50	3007	2975	32	5330	64	1.55	8121	8134	-14	10053	-17		
		2.52	2997	3010	-13	5420	-26	2.41	7352	7337	15	10015	21		
		2.55	2985	2996	-11	5416	-22	2.43	7338	7311	27	9999	37		
								2.46	7320	7283	37	9984	52		
								1.96	7324	7290	-236	10346	-310		
								1.97	7725	7831	-105	10174	-138		
								2.06	7647	7760	-114	10187	-151		
“Kayseri”	4775														
Coal		1.01	4232	4007	226	4507	268								
		1.05	4223	3983	240	4489	286	1.07	4024	3983	41	5628	61		
		1.46	4086	3869	218	4506	269	1.09	4003	3955	48	5615	74		
		1.50	4067	3843	224	4496	279	1.49	3680	3596	84	5548	141		
		1.99	3924	3711	213	4499	276	1.61	3586	3496	90	5533	156		
		2.00	3922	3704	217	4494	281	1.97	3350	3251	99	5504	185		
		2.52	3774	3597	176	4537	238	1.98	3345	3256	89	5523	166		
		2.64	3744	3526	218	4479	296	2.47	3072	2986	86	5511	178		
								2.48	3067	2982	85	5514	175		
“Bor-Mengen”	5794														
Coal		1.00	4119	4064	55	5710	84								
		1.00	4121	4070	51	5716	78	0.99	4210	4139	72	5852	110		
		1.49	3684	3624	60	5691	103	0.99	4213	4211	2	5959	3		
		1.51	3671	3641	29	5743	51	1.46	3791	3775	16	4843	27		
		1.99	3335	3275	61	5677	117	1.50	3744	3628	116	5760	202		
		2.01	3326	3197	129	5546	248	1.50	3744	3689	56	5865	97		
		2.49	3054	2980	74	5638	156	1.95	3422	3363	59	5848	114		
		2.50	3050	2994	56	5675	119	1.95	3419	3339	43	5879	83		
								2.00	3389	3255	134	5702	260		
								2.44	3131	3100	32	5477	67		
								2.49	3099	3046	54	5847	115		
								2.50	3097	3012	85	5780	182		

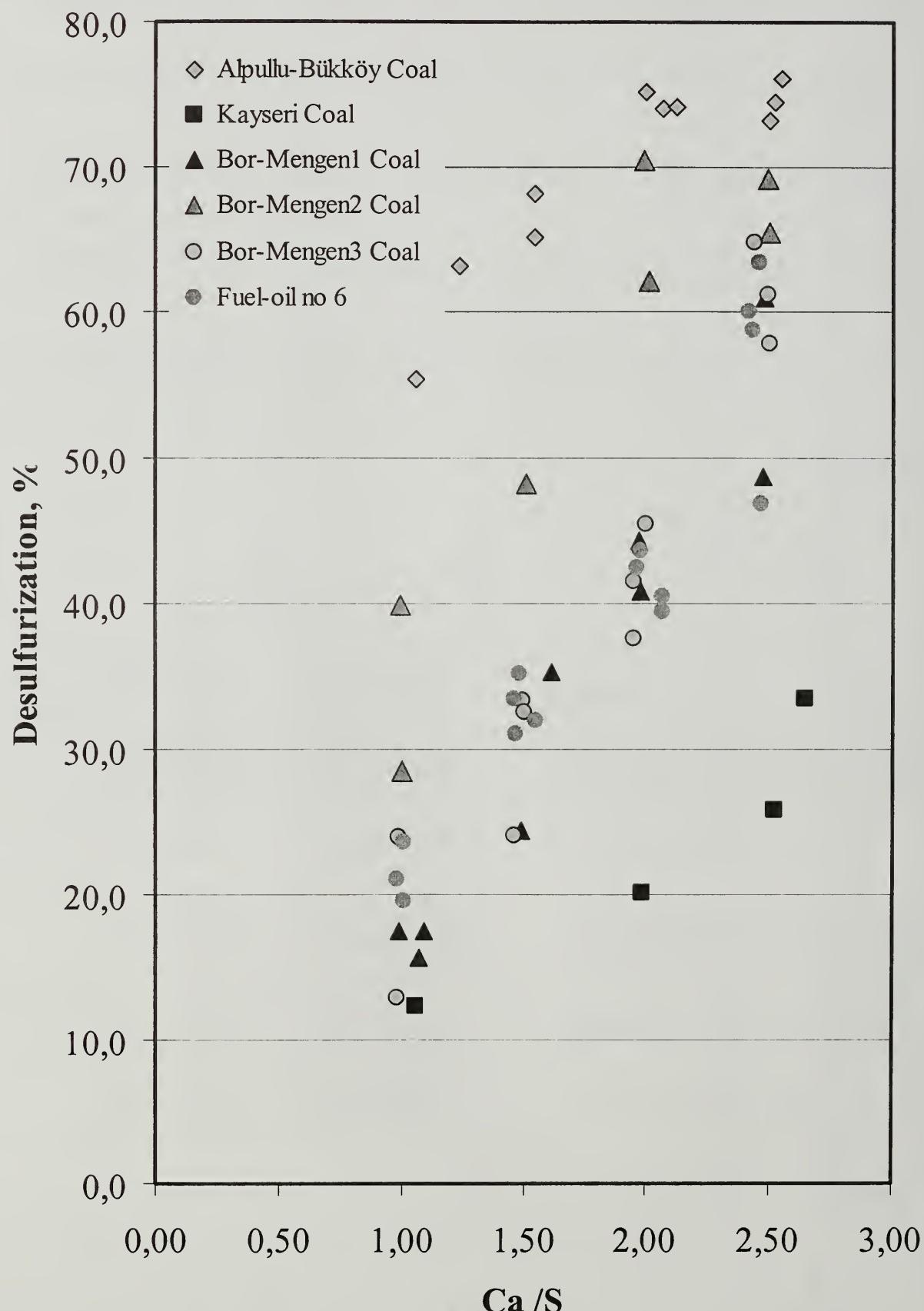


Figure 1. Desulfurization effect of carbonatation sludge addition

REAL TIME SOLUTION COLOUR OF CRYSTALLINE SUGAR

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ABSTRACT

Solution colour is a very important quality property of sugar. Monitoring of sugar colour in real time permits tight control of the process and prevents shipment of sugar outside specifications. Colour determination by the laboratory ICUMSA methods have some drawbacks that can be compensated by in-line measurements in real time. Neltec has developed an instrument for this. The two methods will be compared. A description is given of the instrument's installation, function, applications, and performance with examples of installations on wet and dry sugar. Examples are presented of process optimisation and of detection of error conditions in the centrifugals. Calibration with measurements from the factory laboratory and the resulting accuracy will be discussed. Examples of the accuracy obtained in several installations will be presented. Comments on an eventual collaborative test of this indirect method are included.

INTRODUCTION

For many years the ICUMSA methods¹ for colour in solution have been used to classify sugar. The methods are well known and globally accepted and recognised. However, the methods have some drawbacks. They take time, thus reducing the measurements' usefulness for process control. They have direct costs for chemicals and filters. They involve manual work of qualified personnel. As a consequence, it is not possible to get a large number of measurements at short notice, when you really need them. There are too few measurements too late to achieve tight control of the process. As a result, most refineries run the process on the 'safe side', making too good sugar with a too high consumption of chemicals and energy, bad utilisation of the capacity in the sugar house, and spraying too much in the centrifugals, resulting in too high purity of the molasses.

Neltec has developed an in-line colorimeter to make solution colour measurements in real time. It requires no sample preparation, measures directly on belt, hopper, or scraper conveyors, or in screw conveyors. In all 99 installations the ColourQ instrument has proven its performance and

stability, measuring sugars in the range of 3 to 10,000 IU. (No distinction is made between the ICUMSA methods for colour measurement. The method used by the sugar producer is always used for calibration and test.)

The instrument has been tested by many researchers and sugar producers. (Refs. 2 to 10)

THE INSTRUMENT

The optical part of the instrument is shown in Figure 1. The two tubes of 90 mm stainless steel pipe are the system's illuminator and detector. The illuminator sends out extremely short pulses of light - like a stroboscope. The light reflected from the crystalline sugar is collected and separated in various wavelengths in the detector. In the system's computer the data are processed to determine the solution colour of the sugar.

The environment in a refinery can be hard on sensitive instruments. To prove the system's robustness we asked a customer to let a sugar tanker run over the instrument. It worked before, during, and after the test.

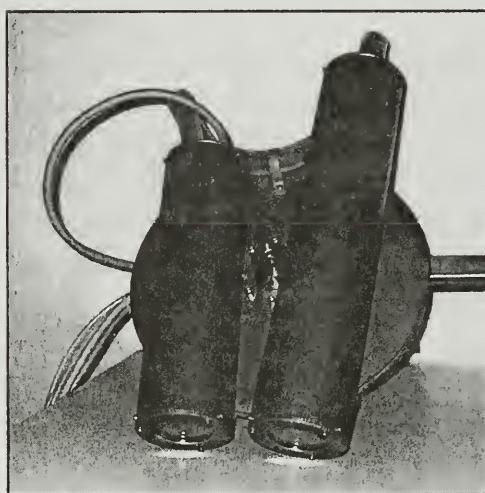


Figure 1. Illuminator and detector, the optical parts of the instrument.

DRY SUGAR MEASUREMENTS

Figure 2 shows an installation on a belt conveyor with white sugar. The results are presented on the computer's screen, as ICUMSA solution colour versus time, figure 3.

With one measurement per second the colour of the sugar is known in good time before the sugar reaches the bin or the silo. Some instruments are installed at delivery and used to print a report of all measurements taken on a load of sugar to prove the quality to the customer.

Measurement on raw sugar is shown in figure 4.



Figure 2. Installation on a belt conveyer with white sugar.

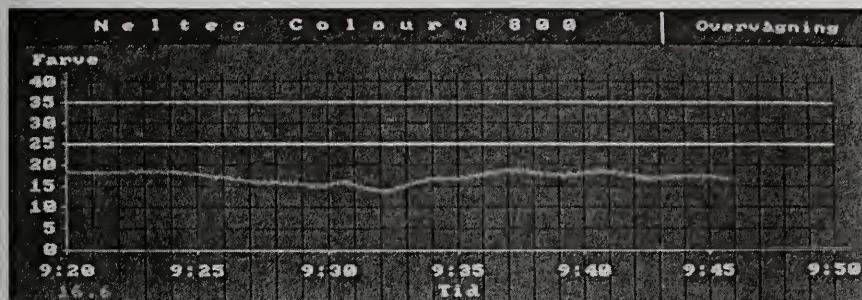


Figure 3. Example of results presented on the computer screen.



Figure 4. Installation showing measurement of raw sugar.

WET SUGAR MEASUREMENTS

Figures 5 to 8 show installations on different conveyors with wet sugar, right after the centrifugals. Here the measurements are very close to the process and can be used for process control.

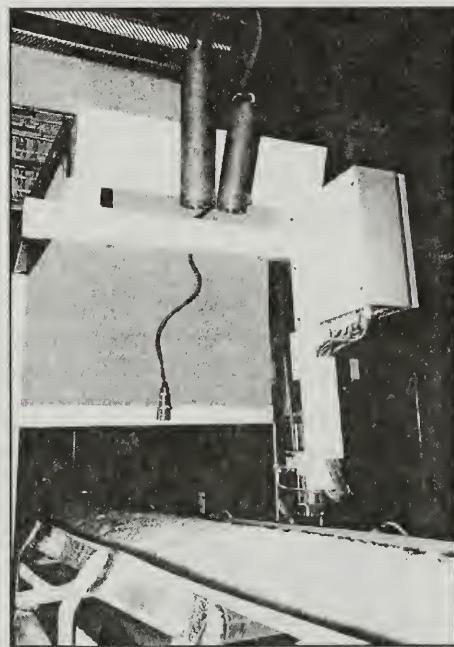


Fig. 5. Wet sugar on belt

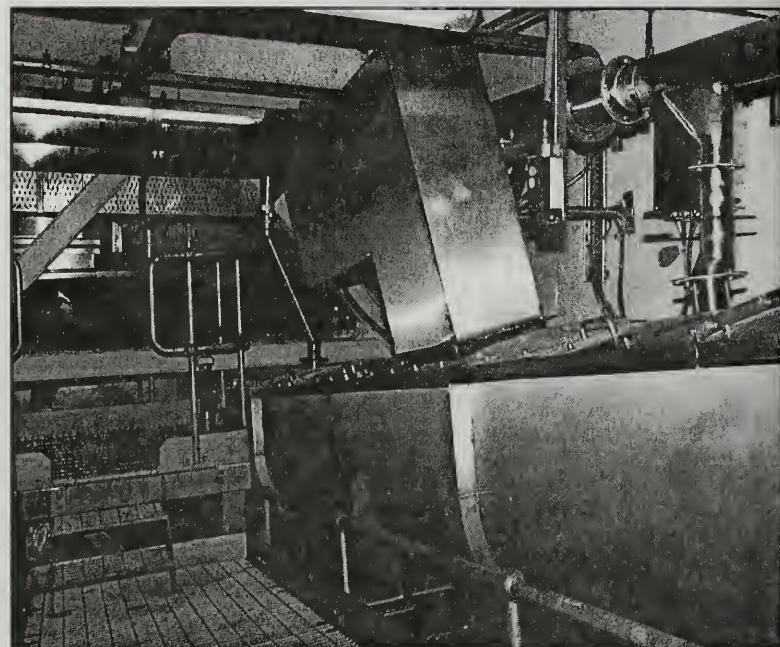


Fig. 6. Wet sugar in screw

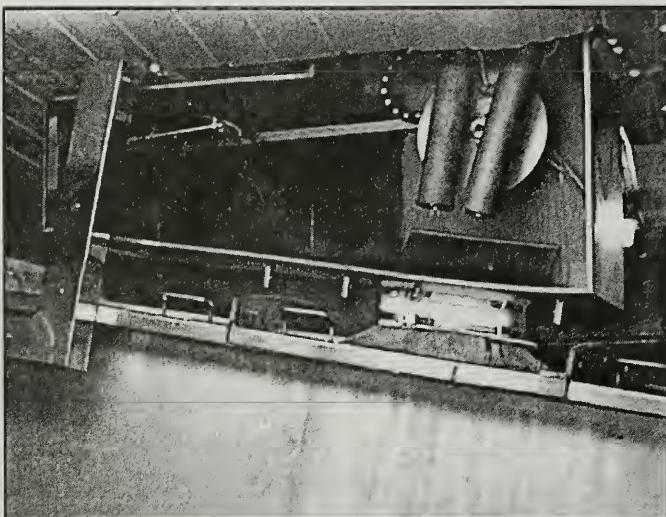


Fig. 7. Wet sugar in screw

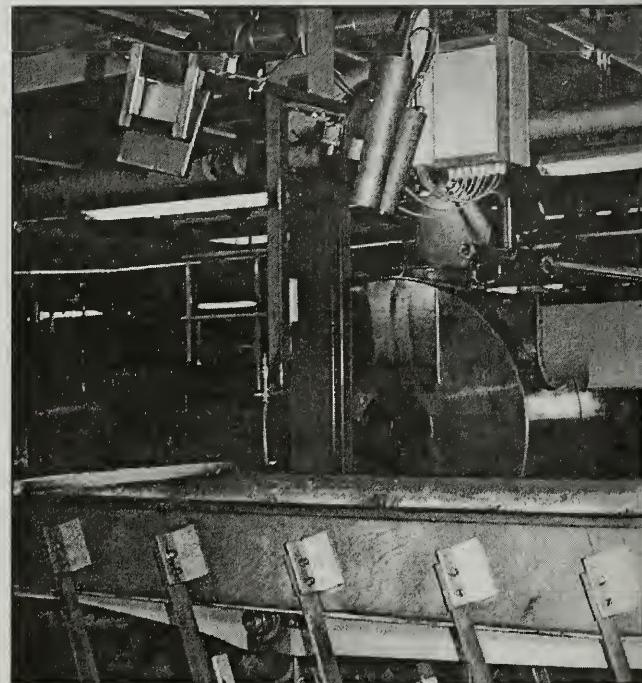


Fig. 8. Wet sugar on grasshopper

OPTIMISATION OF CENTRIFUGALS AND PROCESS

After observing the sugar coming out of the centrifugals at many installations it is evident the process is very dynamic and requires tight control to ensure a uniform quality out of the centrifugals.

Washing in the Centrifugals

The water sprayed on the 'wall' of sugar hits the inner layer of sugar as pure water. On its way out through the sugar, its concentration of sugar increases, reducing its capability to clean the sugar. This means that when the inner sugar crystals are cleaned from the syrup of the massecuite, there is still a layer of syrup on the surface of the outer crystals near the screen. This is illustrated in Figure 9. Spraying with more water is required to clean the outer crystals. This additional water melts good sugar from the surface of the inner crystals on its way to the outer crystals.

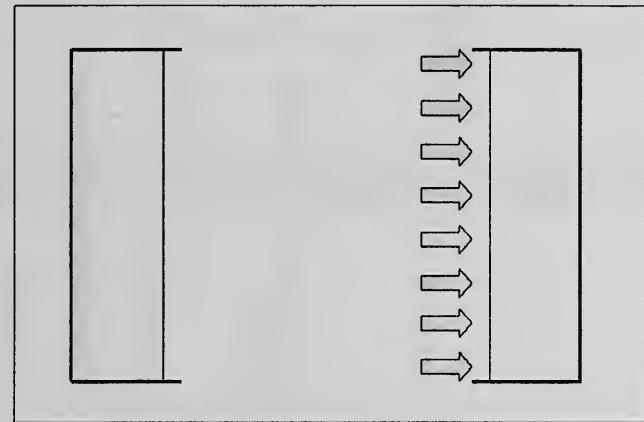


Fig. 9

Figure 10 shows the dilemma for the manager of the centrifugals. How much of the spraying is 'not necessary'? Should a colour increase of 50% from the inner to the outer crystals be accepted? or 5%? or 0.5%? You have to stop at some point, if you want to have some sugar left in the centrifugal.

Exaggerated spraying means that less sugar is going to the silo from the same amount of massecuite, and less sugar than possible is produced with the installed capacity. With any bottlenecks in the refinery, it is quite costly to produce less sugar than possible. By exaggerated spraying more sugar has to be reprocessed - leading to higher energy consumption and loss of some of the reprocessed sugar to molasses.

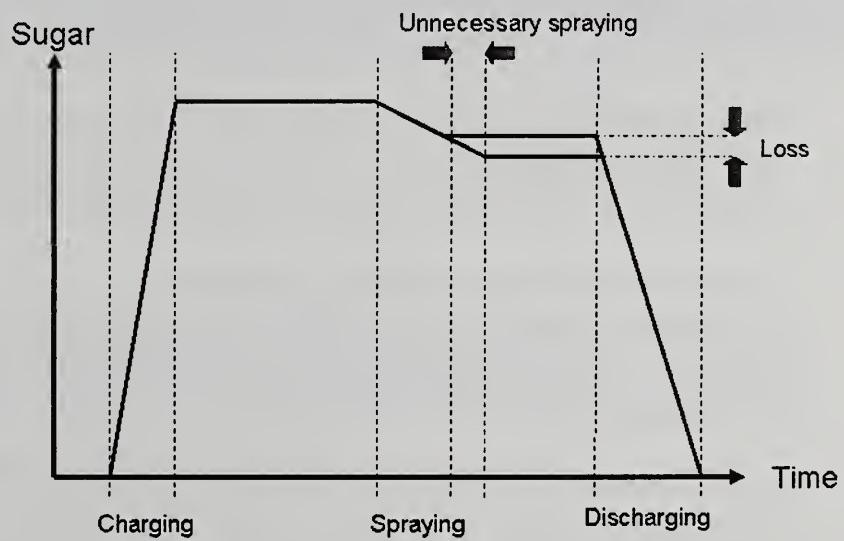


Fig. 10

Ideally, the decision about the amount of spraying water should also take the quality of the massecuite into consideration. If the current pan has massecuite with a colour that is 10% higher than the previous pan, then it might be an intelligent decision to increase the spraying or to reduce the filling of the centrifugals, or both. This might be more cost-effective than remelting the whole pan - or having it in the silo.

Example of Potential for Centrifugal Optimisation

The potential for centrifugal optimisation is illustrated in Figure 11. In this factory, the variation of the sugar colour with variations in the spraying water amount was checked, before the centrifugals had been optimised.

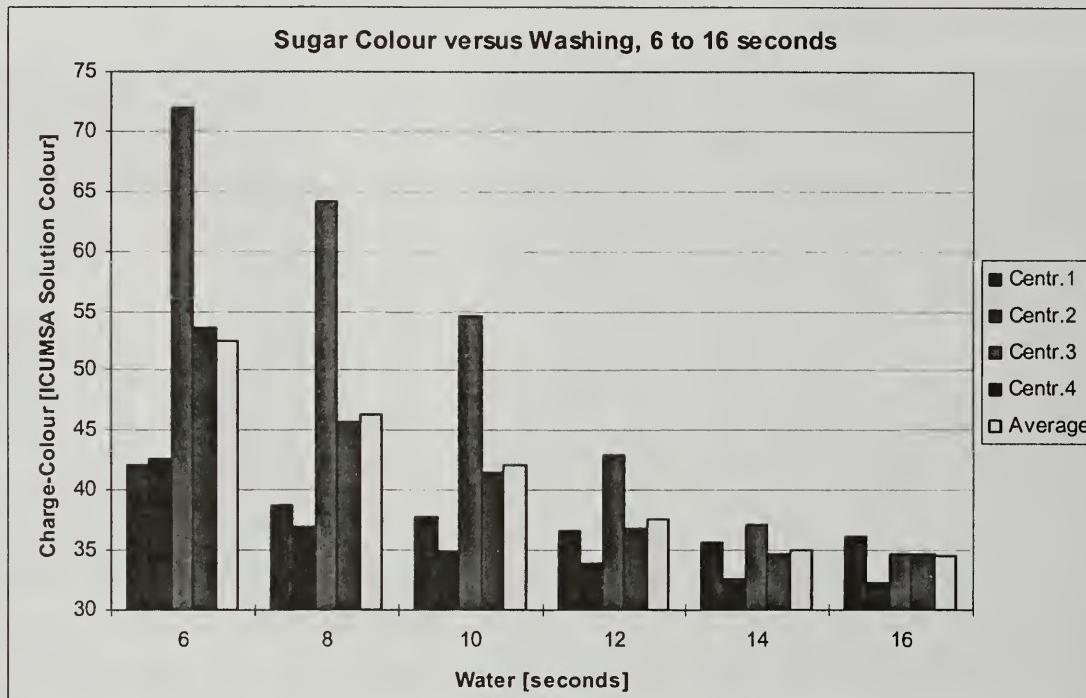


Fig. 11

If the centrifugals had been running with 16 seconds of spraying, then the average colour was 34 IU. If all centrifugals could be brought to run like centrifugal #2, then the same average colour would be achieved with 12 seconds of spraying, only. This is a water reduction of 25%.

Example of Uniform Centrifugal Operation

Optimisation resulting in completely uniform operation of the centrifugals is shown in Figure 12. In the bar on top of each of the four centrifugal windows, you see the average colour from the centrifugals during the last cycle. They are 24.8, 24.5, 24.6, and 24.7 IU, respectively.

In the centrifugal windows you see a colour profile of each charge from the beginning to the end of the charge. The profiles here are almost flat with very low colour increase over the charge. The thin curves at the bottom of the centrifugal windows show the amount of sugar in the screw conveyor. Apparently, centrifugals 1 and 4 deliver double the amount of centrifugals 2 and 3. This is because they discharge on top of each other.

In the large window in the lower part of the screen you see the colour over the last two days. It has been possible to keep the colour within the narrow range from 25 to 32 IU, by timely corrections to the process. The average colour per shift for the current shift and the three previous ones can be seen below the curve. They are 26.9, 27.0, 26.4, and 27.7 IU, respectively.

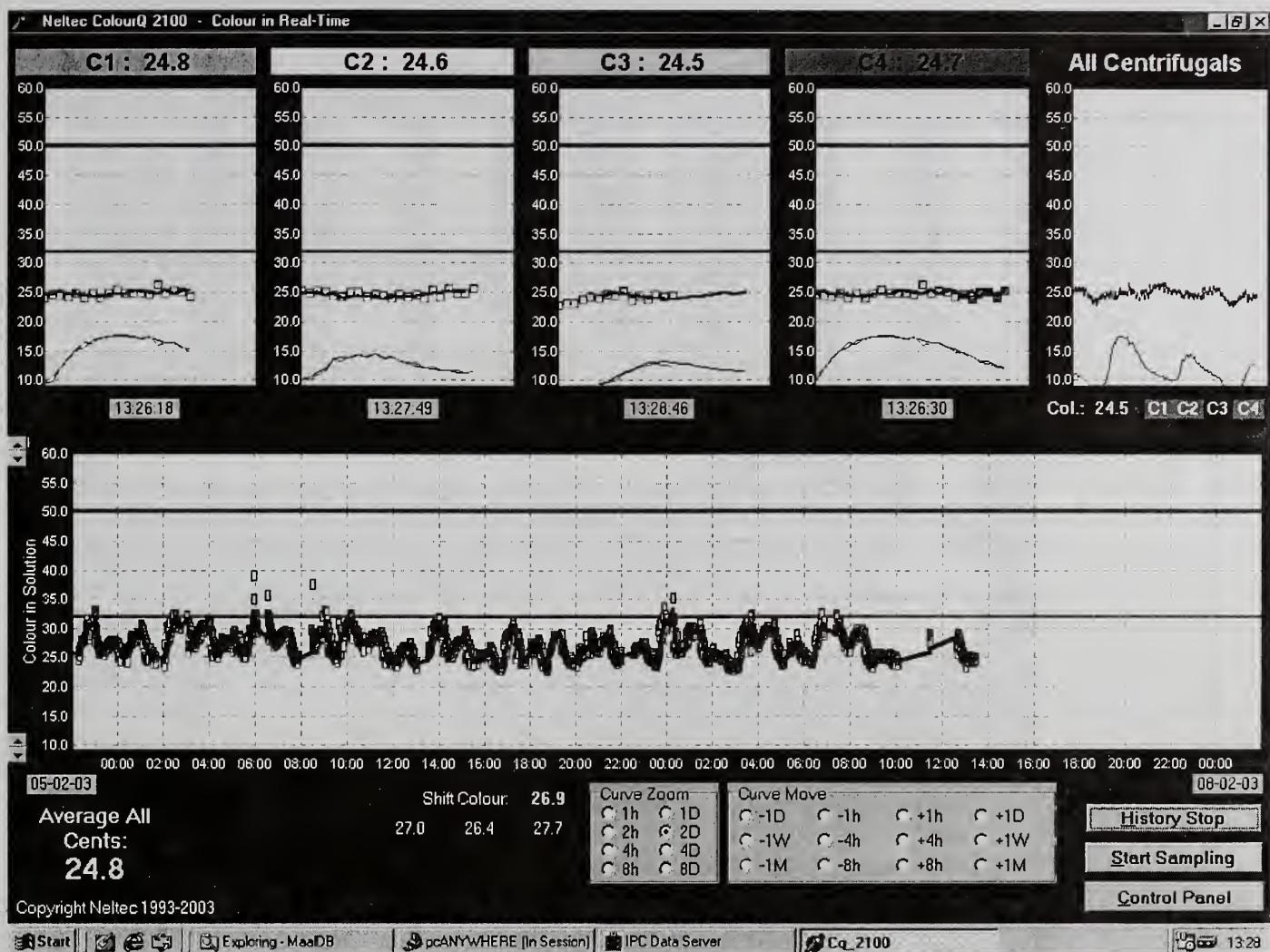


Figure 12. An example of completely uniform operation of the centrifugals.

Example of Process Variation

Sometimes errors outside the process make it impossible to have a uniform operation. In Figure 13 the power was completely lost for a considerable time. After the power came back it took some time to get the colour back to normal. The real time measurements helped decide the best time to switch the sugar from remelting to sending it to the silo.

Example of Centrifugal Error

Figure 14 shows an example of an error in a centrifugal. In this case the error is in the centrifugal #2.

Example of Insufficient Flushing of Screen

In Figure 15 the screen of centrifugal #1 needs flushing. The syrup cannot get completely purged from the basket and raises the colour of the outer layer of sugar, especially near the bottom of the centrifugal.

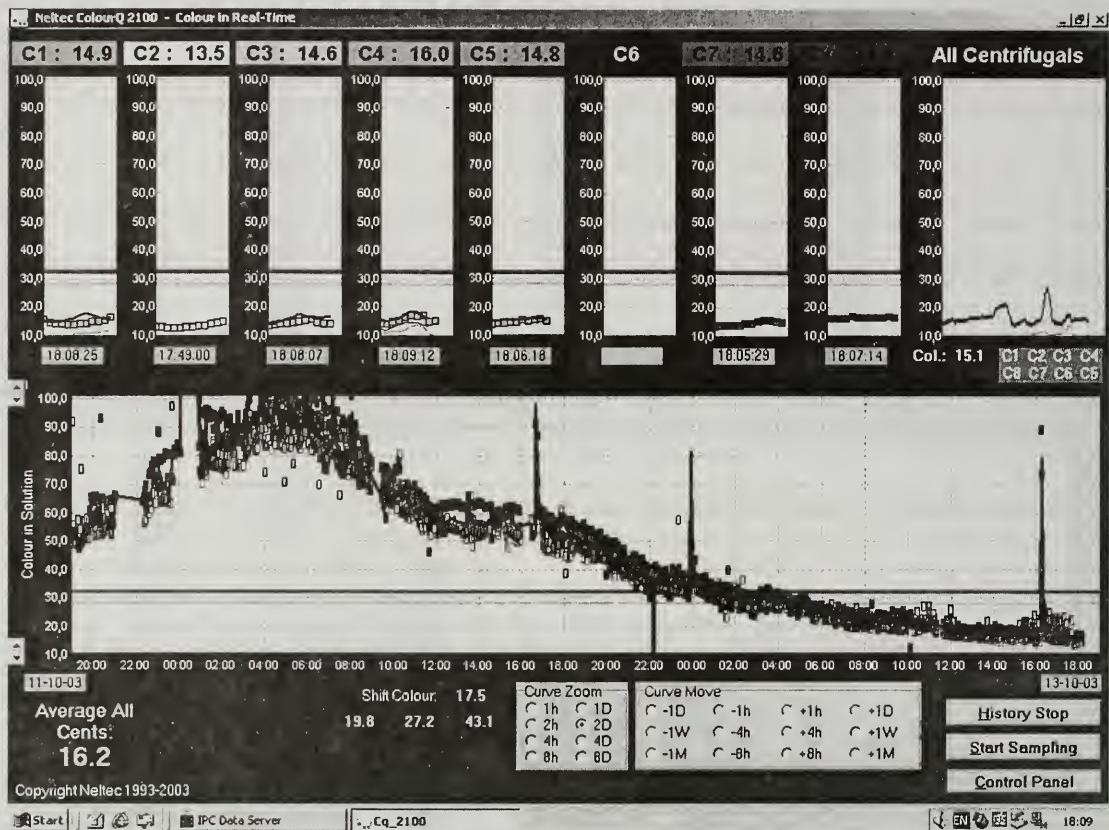


Figure 13. Example of process variation caused by a power failure.

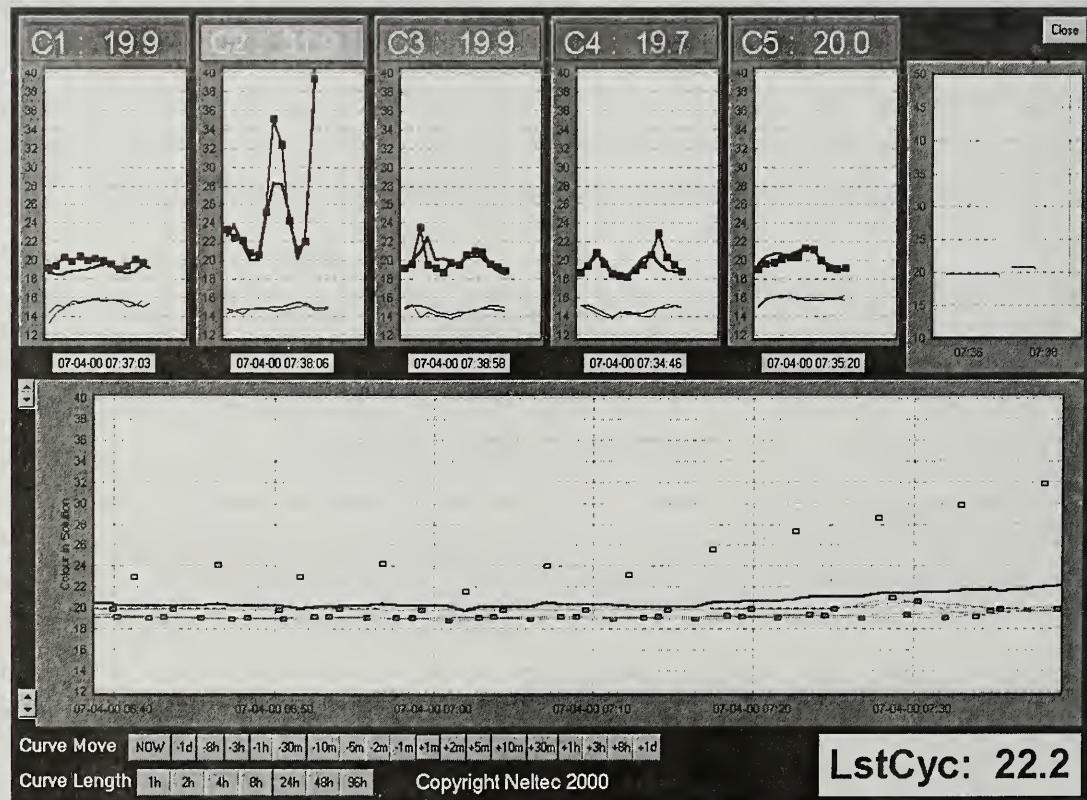


Figure 14. Example of an error in centrifugal #2.

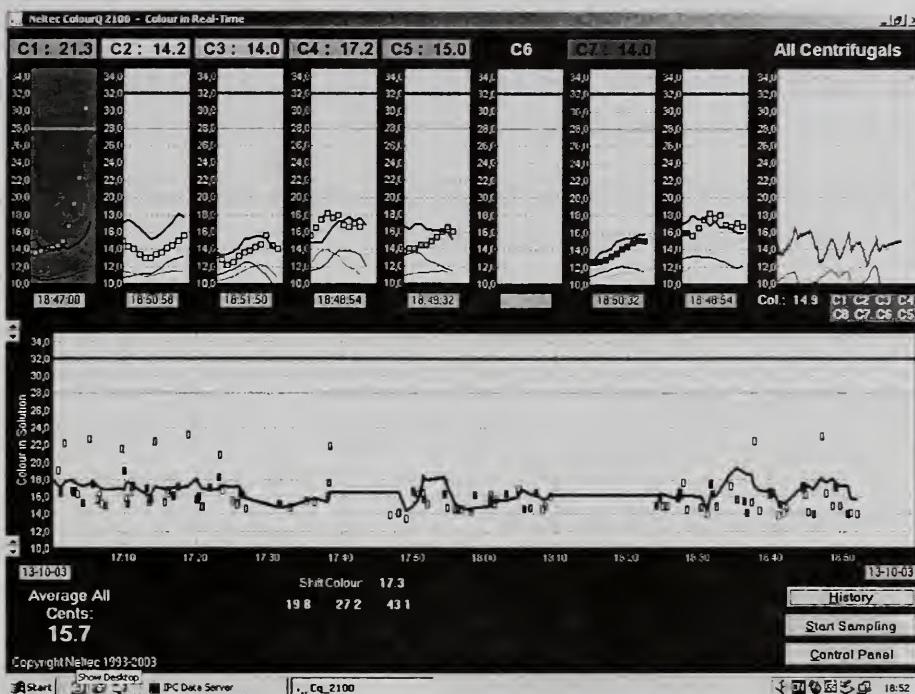


Figure 15. Example showing insufficient flushing of the screen in centrifugal #1.

ACCURACY

The in-line instrument is an indirect method to measure the (laboratory) solution colour. It should give results comparable with the results from the laboratory. A small deviation between the two methods means that the indirect method has a good accuracy. The deviation is measured by the Standard Error of Prediction (SEP). The SEP is calculated by:

$$\text{SEP} = \sqrt{\left(\sum_{i=1}^N (\text{Colour}_{\text{Laboratory}} - \text{Colour}_{\text{ColourQ}})^2 \right) / N}$$

The errors that may contribute to the SEP are:

- Errors from the ColourQ Instrument
- Errors from Sampling
- Errors from the Laboratory Determination

Errors from the ColourQ Instrument

You would expect a difference between the two methods because the ColourQ makes:

- No pH-adjustment
- No filtration
- Reflection instead of transmission

Errors from Sampling

If the sugar measured in the laboratory is not exactly the same as measured in-line, then there is a sampling error. The sampling error is influenced by:

- The conveying method. In a screw conveyor sampling is much more difficult than on a belt.
- If the sugar is dry or wet. Dry sugar has been blended and made uniform in the dryer, while wet sugar has variations from one second to the next.

Errors from the Laboratory Determination

The measurements in the laboratory are influenced by:

- Variations in the laboratory spectrophotometer.
- Filter variations.
- De-aeration (or not).
- Operator skill variations.
-

Example of Accuracy - 1

The most comprehensive test of the ColourQ accuracy reported has been done by Mr. Laurent Bienaimé at the Tereos sugar factory in Origny, France. Here the instrument was placed over a belt with white dry sugar. During more than a year the instrument was compared frequently with the lab measurements. The total SEP over all measurements was better than 1.4 IU. The results were presented⁷ to the C.I.T.S. conference in 1999. In his summary Mr. Bienaimé wrote: "Compared to the laboratory, the additional inaccuracy introduced by the instrument is so small it is negligible".

Example of Accuracy - 2

This check was done on a system measuring dry white sugar on a belt. Here the factory lab could not do the necessary measurements for the calibration, so the samples were sent to an independent lab for measurement. After the calibration had been installed, the instrument was checked by a set of samples that were measured by both the factory lab and the independent lab. The results can be seen in Figure 16. The independent lab and the ColourQ have a small deviation, whereas the factory lab has larger deviations to the other results. Analysis of the data showed that the difference was due to two measurements from the factory lab with large deviations.

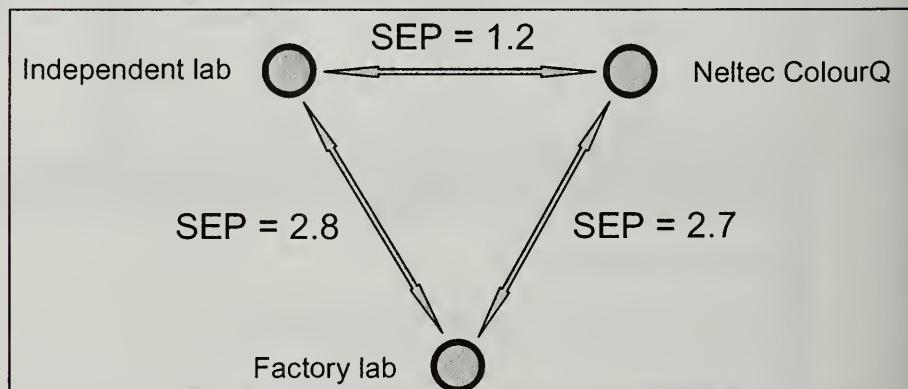
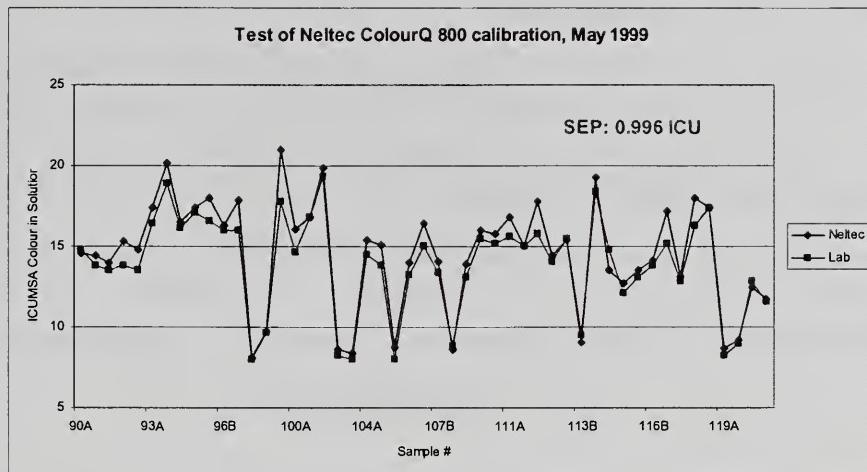


Fig. 16

Example of Accuracy - 3

This check was done on a system measuring wet white sugar on a grasshopper conveyor. The results can be seen in Figure 17. The SEP is smaller than 1 IU.

Fig. 17



Example of Accuracy - 4

This check was done on a system measuring dry white sugar in a screw conveyor. The results (fig. 18) were not satisfactory.

To get a clue to the reason, we agreed the central lab should make repeat measurements on the samples. The large deviation between factory and central lab shows there is a problem in the lab measurements. When the lab has a problem, then the ColourQ has a problem. It can never get better results than the lab, because it is calibrated and checked by the lab.

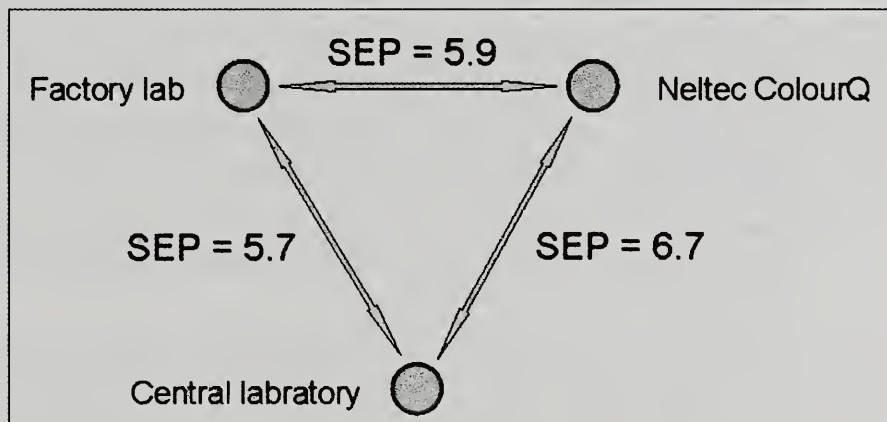


Fig. 18

In such a case the lab must review its procedures and the calibration procedure must be repeated.

Accuracy Conclusion

- The ColourQ can deliver results that add no significant error to the lab error.
- The ColourQ calibration and SEP are much influenced by errors at sampling and in the laboratory.

COLLABORATIVE TESTING

If collaborative testing should be considered for an instrument and method as described here, then the following points are important:

- The results depend on the way the sugar is presented on the conveyor. The angle and distance will be different, if the sugar is not presented like the normal production stream, leading to deviating results. This means a test cannot be done with samples from other sources than the production stream.
- The results depend on the results measured by the local lab on the samples used for the calibration of the instrument. Therefore, a test should include a test of the local laboratory.

SUMMARY

In summary, it is possible to measure solution colour in real time by an indirect method. Monitoring of the process in real time leads to improved quality and reduced costs.

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PROCESS AND ENERGY OPTIMIZED BEET SUGAR FACTORIES

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ABSTRACT

The technological processes of a modern beet factory require continuous reconsideration and development, priority being given to saving of primary energy, improvement of quality and consideration of more stringent environmental regulations. More optimization means correlations become increasingly complex and can only be looked at in all their details.

Examples of modern beet factory models will be presented considering especially optimized parameters for the main process steps, major developments like steam drying and/or continuous crystallization and their impact on the overall factory concept.

INTRODUCTION

Whether or not a beet sugar factory operates efficiently is determined by a number of factors. One of these is the amount of energy consumed. However, not only a cut in costs directly incurred by the fuel used is gaining significance, but also a reduction in the pollutant emission, both in terms of total amounts and concentrations.

When beet sugar factories are to be optimized for energy conservation, this generally affects the complete factory concept. In how far specific measures will prove to be efficient under given boundary conditions remains to be investigated for each specific case. The underlying concept should, however, always be based on long-term considerations and should be implemented on a step-by-step basis.

The chapters below present a realistic basic model, starting from which a number of measures are presented that aim at optimizing energy consumption in the main process. A final chapter will take a closer look at how conversion of the pulp drying process to steam drying may affect the energy efficiency.

BASIC FACTORY MODEL

Before a factory can be optimized, a detailed analysis has to be made of the current situation. This includes listing the details of the available equipment, determining the process data from control system data, and the laboratory data. This then serves as the foundation for preparation of the basic model with a complete mass balance. Special program tools that have been developed for the purpose may be used for plausibility checks of the data thus established.

The basic model is shown in the form of the schematic diagram in Figure 1.

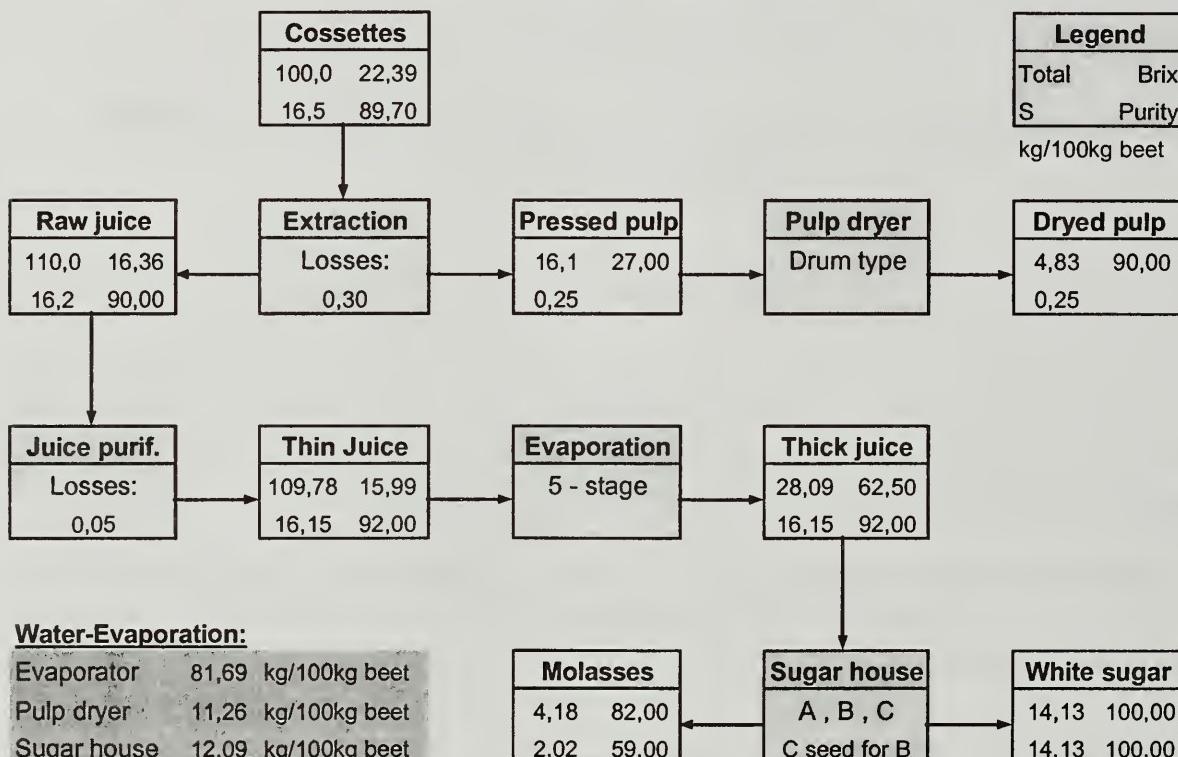


Figure 1. Overall mass balance.

The basic model is characterised by the following elements:

- Extraction tower with countercurrent cossette mixer, draught 110 kg/100 kg beets
- Pulp presses for 27 % pressed pulp dry matter
- Classical juice purification (Pre-Liming, Main Liming, 1st and 2nd Carbonatation)
- 5-effect evaporator station, effects 1&2 Robert evaporator, effects 3-5 falling-film evaporator
- Sugar house with White, High Raw and Low Raw crystallization; White without crystal seed production, Low Raw sugar as crystal seed for High Raw massecuite, hence no Low Raw sugar affination
- Conventional pulp drying, directly fired drum dryer

Details of the sugar house mass balance are depicted in Figure 2.

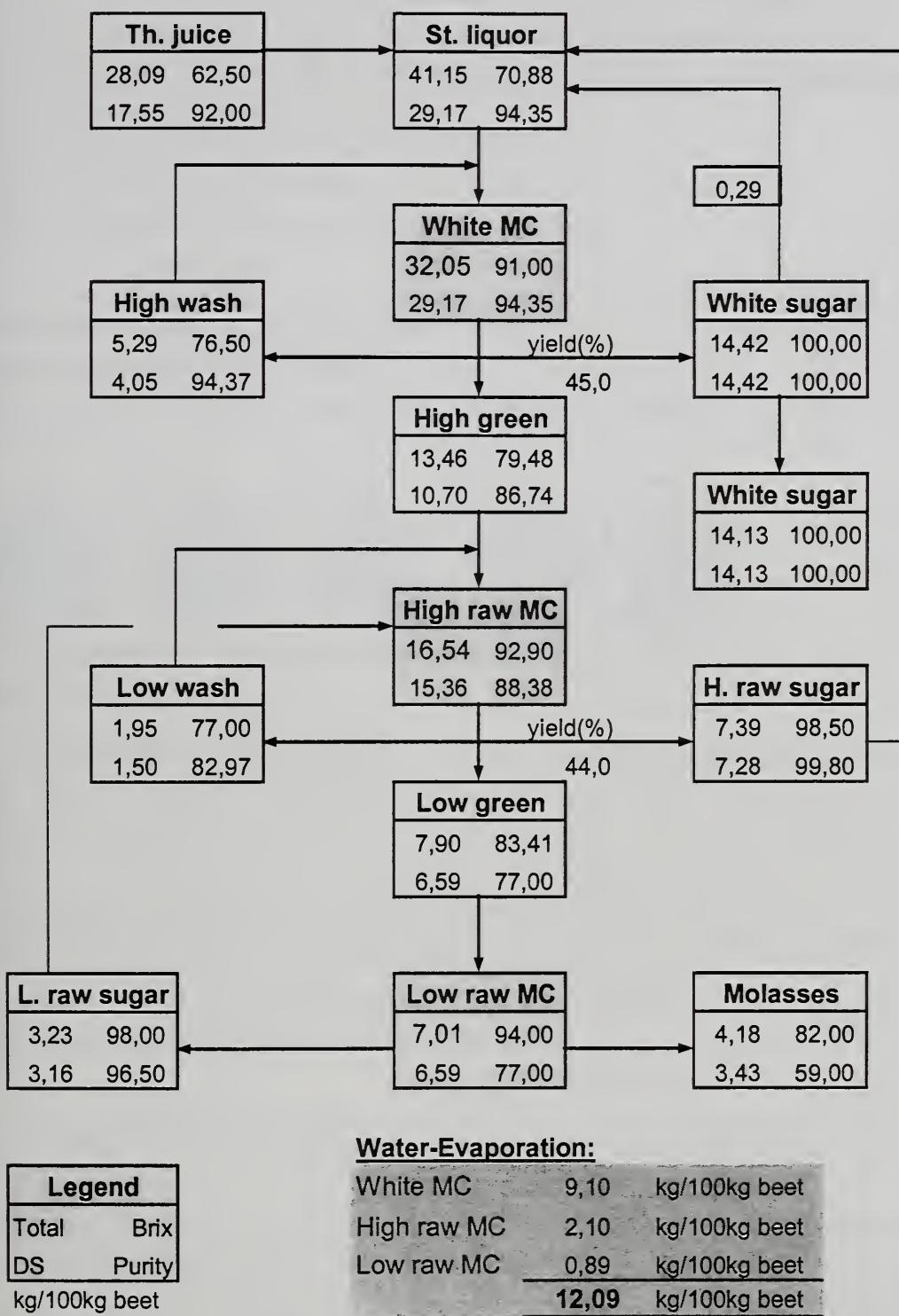


Figure 2. Mass balance of the sugar house.

The heat balance (Figure 3) considers the following users:

- 5th vapour: heat exchanger extraction, pre-limed juice
- 4th vapour: heat exchanger clear and thin juice, pre-limed juice, standard liquor, high raw vacuum pans
- 3rd vapour: heat exchanger thin juice, white and low raw vacuum pans

- 1st, 2nd vapours: heat exchanger thin juice
- Exhaust steam: heat exchanger thin juice, sugar drying
- Condensate 4B: pre-limed juice and raw juice
- Barometric water: raw juice

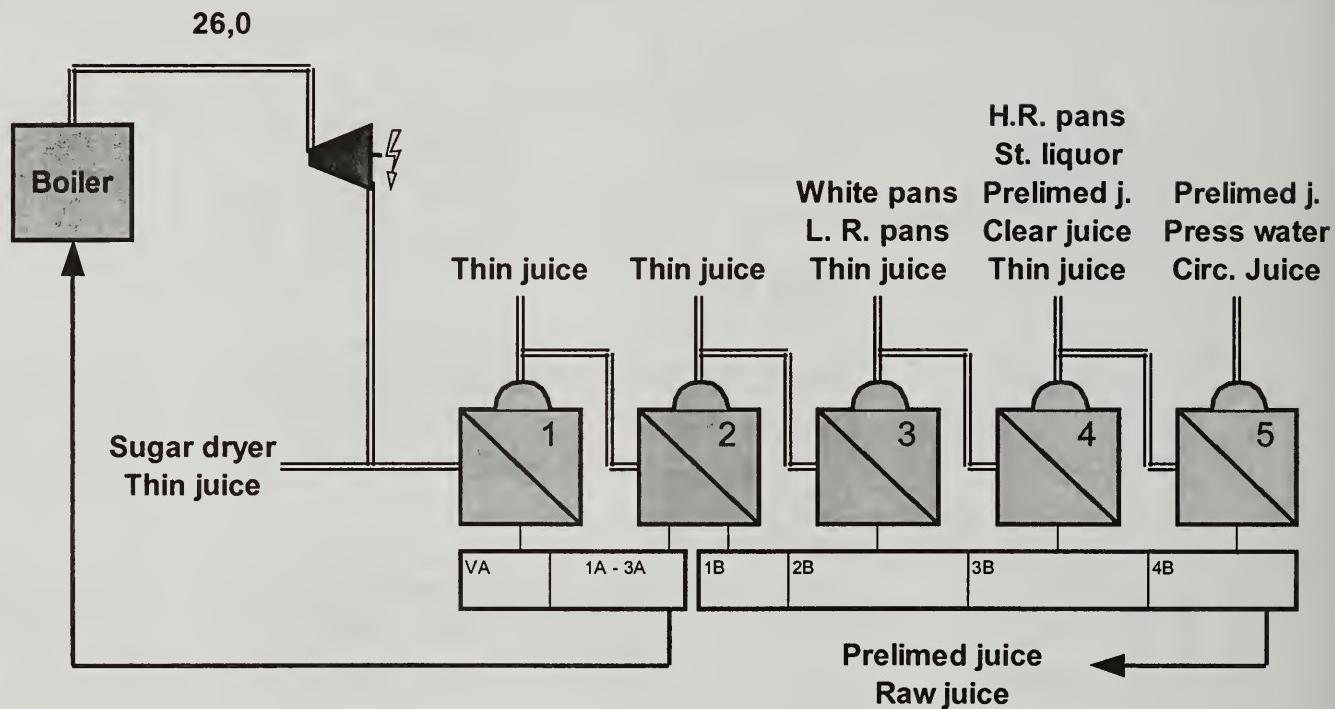


Figure 3. Heat balance.

The boiler house is intended to supply a 46-bar life steam pressure at an assumed efficiency of 85 %. The condensate to the boiler house is supplemented by 1st vapor, and it is depressurized to the 3rd effect pressure before it is returned to the boiler house. Up to a boiler pressure as specified, this method is still commonly used. In this way, sufficient power can be generated to supply the basic model with the required electric power.

Characteristic data for the basic model, starting from a beet processing rate of 10,000 mt/d:

- Energy demand of boiler: 242 kWh/t b.
- Live steam: 26.0 kg/100 kg beets.
- Electric power generated: 14.6 MW
- Exhaust steam pressure to 1st effect: 3.0 bar
- Heat exchanger surface required: approx. 2,500m²
- Evaporator surface required: approx. 16,900m²

For calculation of the pulp drying system it was assumed that the boiler flue gases are utilized, and that the exhaust gas is recycled. This means that under the conditions shown in Fig. 1, a total of 95 kWh/t beets of energy will be required. This, in turn, implies that the total energy requirement of the factory is 337 kWh/t beets.

IMPROVEMENTS IN STANDARD PROCESS OPERATIONS

The options available for improvement of the main process can be broken down as follows:

- Improved utilization of residual heat in condensate and pan vapours
- Dry matter of thick juice raised
- Raw juice draught reduced to 105 %
- Sugar house yield raised
- Dry matter of pressed pulp raised to 30 %

The residual heat of the condensate can generally be utilized in an optimum manner when using condensate to preheat the pre-limed juice leaving the pre-liming system or the cold main liming system to a maximum level. The relevant factor in this process is the difference in temperature between the juice entering the system and the exiting condensate. This difference should not be greater than 5 K, a value which modern plate-type heat exchangers have no problem in attaining.

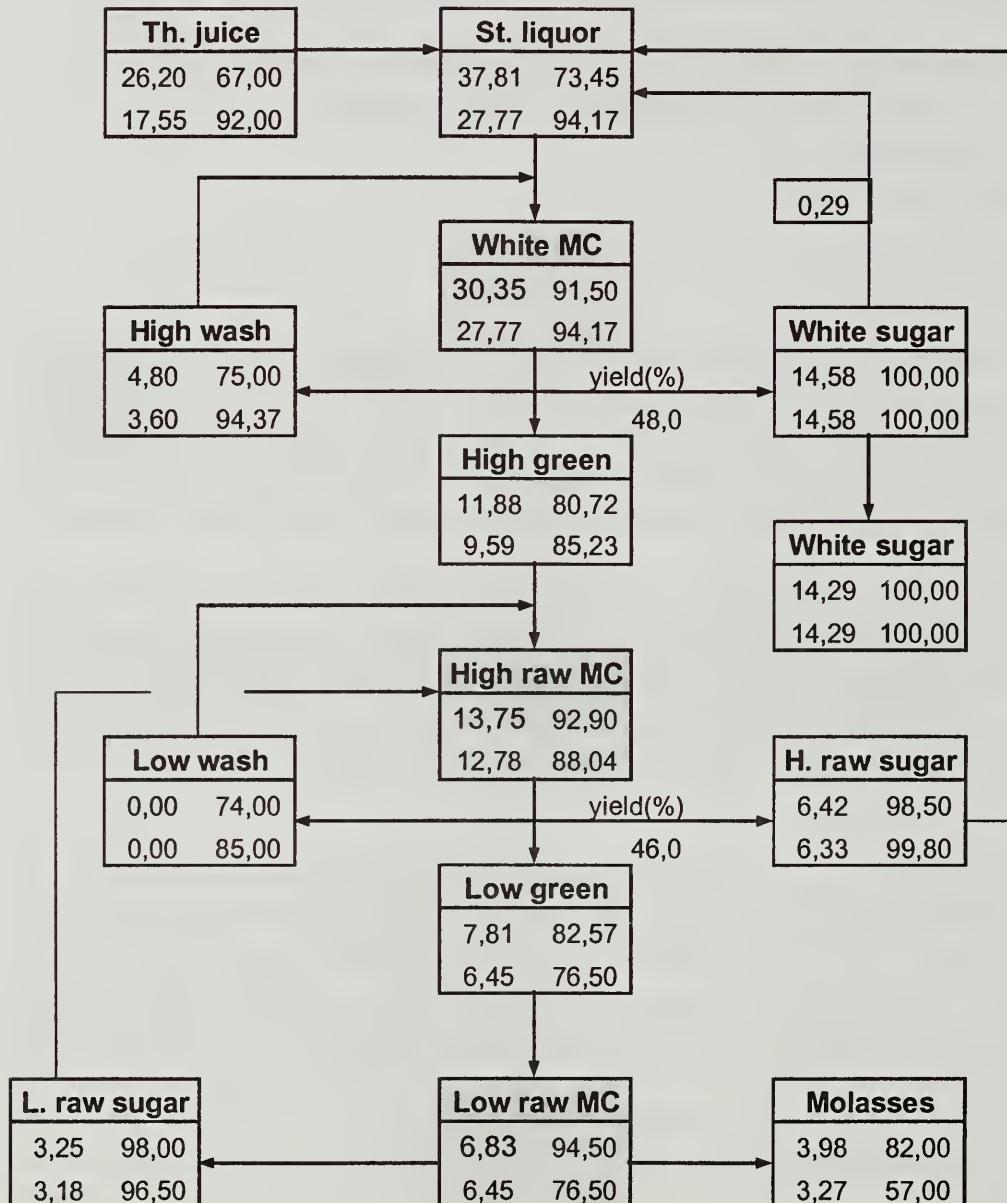
Utilization of sugarhouse vapours should also be optimized with respect to the raw juice temperature. A raw juice temperature of 53 – 55 °C is a target that can be achieved with the systems available today. Possible solutions are either heat exchangers (of the plate or tube bundle type) directly heated by pan vapours, or a pre-condenser producing barometric water that is used to heat a plate-type heat exchanger. Which option offers most cost benefits has to be decided from case to case.

The dry matter in the thick juice can be increased by converting the evaporator station to a 6-effect system. Another factor that requires this conversion to be made is that it at the same time reduces the steam consumption in the sugar house. To avoid condenser losses, the raw juice draught is reduced. As a consequence, adequate measures have to be taken at the A-station end to make sure that crystallization proceeds as required on standard liquor of a raised dry substance content.

The effects of the raised thick juice dry matter and additional measures taken to increase the yield are shown in Figure 4.

The measures designed to improve crystallization, and consequently sugar house yield, include the following:

- Pan seeding for White product as a single- or a two-stage system (as from MA > 0.55-0.6 mm), as required for the intended product crystal size, to improve centrifugal work
- Introduction of syrup washing for crystal sizes MA > 0.4-0.5 mm (this is, however, on the condition of highly uniform crystallization)
- Use of modern crystallizers with stirrers for all products. For High Raw and Low Raw product, continuous systems operating on 6th vapour are employed. The use of 6th vapour also for White massecuite boiling offers special advantages in terms of heat economy. This does, however, not apply to extremely fine product of 0.3-0.4 mm. That's why, optimization calculations consider modern discontinuous equipment for this case. A vapour supply of 60 % 4th vapour and 40 % 3rd vapour was considered here.

**Water-Evaporation:**

Legend			
Total DS kg/100kg beet	Brix	7,46	kg/100kg beet

White MC	7,46	kg/100kg beet
High raw MC	1,38	kg/100kg beet
Low raw MC	0,99	kg/100kg beet
	9,82	kg/100kg beet

Figure 4. Mass balance sugar house

For this optimized case, there will be the following users of thermal energy (Figure 5):

- 6th vapour: heat exchanger extraction, pre-limed juice, High Raw and Low Raw CVP
- 5th vapour: heat exchanger clear and thin juice, pre-limed juice
- 4th vapour: heat exchanger thin juice, White and Low Raw vacuum pans for crystal seed, White pans, standard liquor, sugar drying

- 3rd vapour: heat exchanger thin juice, White vacuum pans for product
- 1st, 2nd vapours: heat exchanger thin juice
- Condensate 5B: pre-limed juice and raw juice
- Barometric water: raw juice

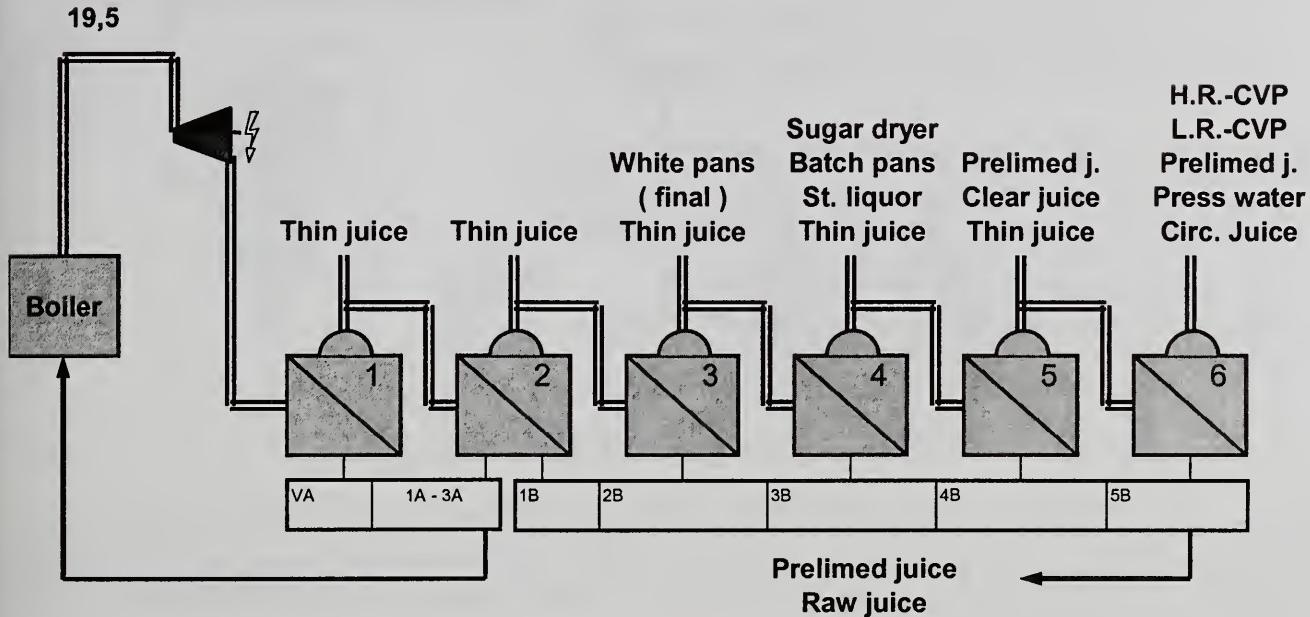


Figure 5. Heat balance optimized model.

The boiler house and turbine are designed as specified for the basic case. This is not sufficient, if the factory is to produce its own electric power. A detailed power supply analysis will, however, not be made at this point. As a general guideline, forward-looking overall concepts would require the boiler house to be designed for maximum pressures. Common boiler pressures are those of up to 100 bars.

A decisive contribution to energy saving measures is the partial conversion of the A-product pans to 4th vapour. This is not least possible because the higher dry matter in the thick juice at the same time reduces the required water evaporation rate. The critical phase of crystal formation is in that case covered by the production of crystal seed.

Characteristic data for the optimized model, starting from a beet processing rate of 10,000 mt/d:

- Energy demand of boiler: 181 kWh/t b.
- Life steam: 19.5 kg/100 kg beets.
- Electric power generated: 11.2 MW
- Exhaust steam pressure to 1st effect: 3.0 bar
- Heat exchanger surface required: approx. 4,100m²
- Evaporator surface required: approx. 22,500m²

Pulp drying has been included in the considerations as specified for the basic model. The higher dry matter of the pressed pulp means that energy is required at a rate of 82 kWh/t beets, which results in overall thermal energy requirements of the complete factory of 263 kWh/t beets.

A comparison with the basic model shows the following differences:

- Life steam rate reduced by 25 %
- Energy required for pulp drying reduced by 14 %
- Overall thermal energy requirements reduced by 22 %

To achieve this, the following essential measures are required:

- Additional heat exchanger surface: approx. 1,600 m²
- Additional evaporator surface: approx. 5,600 m²
- Reconditioning/expansion of the pan station
- Modified heating steam supply for sugar drying

IMPACT OF STEAM DRYING

Pulp drying is one of the key factors for sugar factory energy concepts. This is because, on the one hand, about 30 % of the thermal energy go into directly fired systems, while the exhaust gas leaving the drying station, on the other hand, accounts for a considerable portion of the emission produced by the factory. Many countries are enacting more stringent environmental protection requirements, which make it necessary to invest increasing amounts of money into emission control.

A major field of application for pressed-pulp steam drying in the fluidized bed was the restructuring programme launched for the sugar factories in the new federal states of Eastern Germany in the early 1990s. Since then, this technology has seen decisive developments. Systems now successfully in operation allow – subject to the marc content – even beet processing rates of up to 12,000 mt/d to be handled by a single unit. Aspects to be considered are, however, not only the water evaporation rate, but also the dry matter that needs to be handled. The higher the pressed pulp dry matter, the more decisive is pulp conveyance for plant design.

The following paragraphs take a closer look at the influence of a steam dryer integrated into the system when using the optimised factory model presented above as a basis. Fig. 6 is a schematic view of the integration into the energy concept of a factory.

Implementation of a steam dryer as shown in Figure 6 plays a decisive role with respect to a considerably reduced life steam rate available for electric power generation. On the other hand, the primary energy required for pulp drying can be left out of account almost entirely.

The example illustrated starts from the simplest case: only the portion of the exhaust steam that is directly passed to the evaporator station is used for power generation. Depending on the boiler house and turbine rating, a conceivable option is to use either a specifically designed tapped turbine, or a separate turbine for the part-stream taken to the steam dryer. For a complete island

solution it will, however, normally be necessary to use a gas turbine with waste-heat recovery boiler. To arrive at an effective solution it will always be necessary to establish the required capital expenditure and the operating costs for the specific case in question.

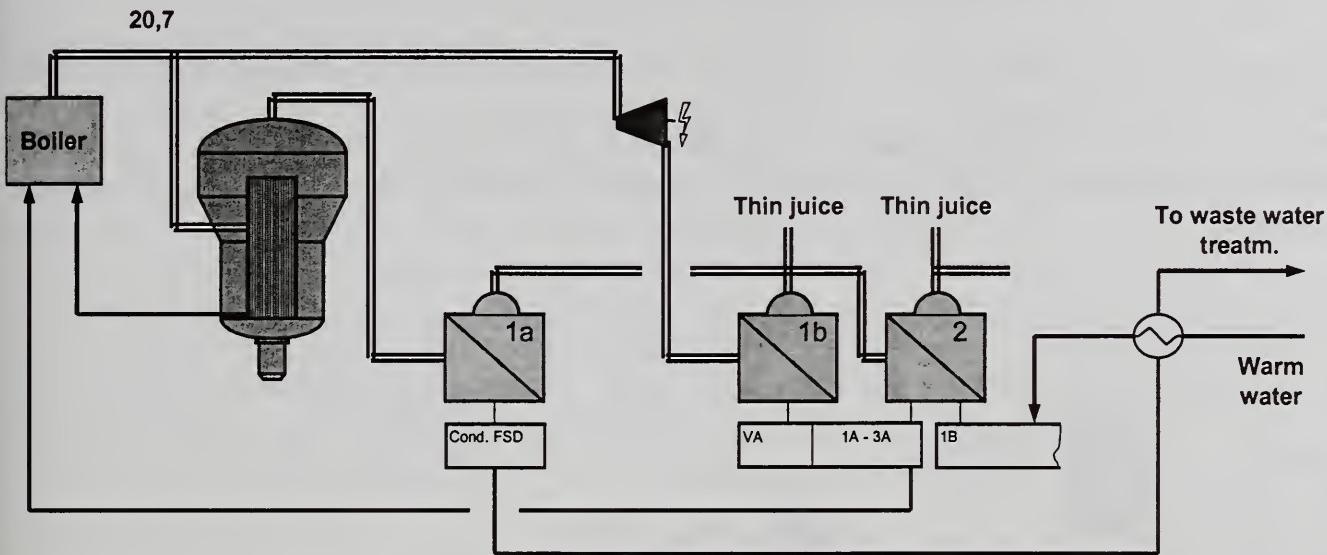


Figure 6. Implementation of a steam dryer.

Another requirement is that the drying vapours have to be used separately. The following options are available:

- Steam transformer between steam dryer and 1st evaporator effect
- 1st effect split up into two evaporator bodies (falling-film/plate-type evaporator)
- Plate-type evaporator with calandrias arranged on top of each other and a common vapour chest

In all other respects, the evaporator station is for this case identical with the system presented for the optimised case. What is added is the cooling of the contaminated condensate produced by the steam dryer vapours with hot water which is then passed into the condensate 1B compartment. This measure provides for additional condensate expansion. To minimise condenser losses that may result from this measure, the A pans have to be completely heated on 4th vapour. If pans with sufficiently large heating surfaces are provided, this can fairly easily be achieved. But even under these conditions, this version still goes along with some slight condenser losses.

Characteristic data for the optimized model, starting from an integrated steam dryer and a beet processing rate at 10,000 mt/d:

- | | |
|---|------------------------------|
| • Energy demand of boiler: | 191 kWh/t b. |
| • Live steam: | 20.7 kg/100 kg beets |
| • Electric power generated: | 5.2 MW |
| • Exhaust steam pressure to 1 st effect: | 3.0 bar |
| • Heat exchanger surface required: | approx. 4,600m ² |
| • Evaporator surface required: | approx. 22,500m ² |

This means that the total thermal energy required will be reduced by 43 % when compared to the basic case, and by 27 % when compared to optimized case.

COMPARISON OF ENERGY DEMANDS

As compared with the basic model a significant reduction in the energy demand is observed by optimization and integration of a steam pulp dryer (Figure 7). The total energy demand comprises the energy demand of the boiler as well as that for the pulp drying. In the basic model a total energy demand of 337 kWh/t b. was determined out of which 95 kWh/t b. came from the pulp drying.

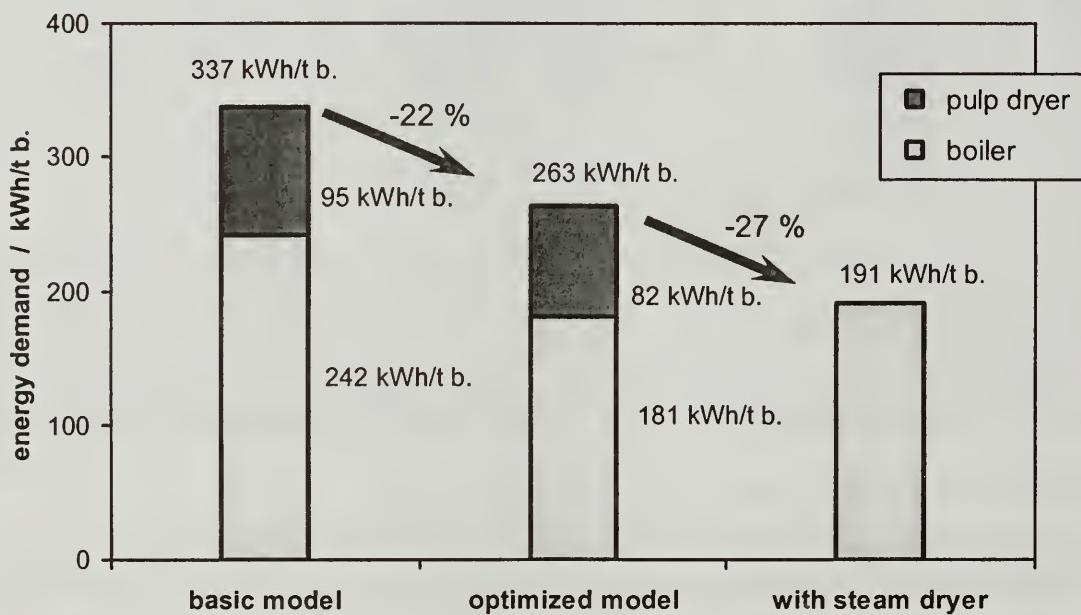


Figure 7. Energy demand of described models.

By optimization of the heat economy and of the crystallization as well as by increasing the dry substance content of the pressed pulp a reduction of the total energy demand by 22 % down to 263 kWh/t b. was stated. Out of these savings 13 kWh/t b. have been contributed by the pulp drying. The integration of a steam pulp dryer leads to a further reduction in the total energy demand in the range of 27 %. The energy demand of the pulp drying is already included in the total of 191 kWh/t b. as shown for the boiler.

CONCLUSIONS

The examples shown illustrate the development of a beet sugar factory characterised by average energy requirements until it reflects the design of an optimised factory with modern pulp drying. Since there is an increasingly closer relationship between product flow and integrated energy concept, planning has to start from more and more detailed calculations. The results have been presented in this paper.

The options available for cutting energy consumption are closely related. This is why one isolated measure often does not produce the intended effect, and only the combined action of a number of measures will mean real progress. Whenever capital is invested, this should, therefore, be done on the basis of a long-term concept. This will not only limit the capital expenditure, but will also ensure that the factory is prepared to meet future requirements. More and more stringent environmental obligations and rising energy costs will have a decisive influence on developments in the beet sugar industry. To be prepared for this by developing the right concepts at an early stage means to be able to survive in a climate of fierce competition.

POSSIBILITIES FOR ION EXCHANGE SOFTENING OF CANE CLARIFIED JUICE

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ABSTRACT

Softening of sugar juices and molasses has become an established unit operation in the beet sugar industry. Being a necessary prerequisite for molasses chromatographic separation technology, thin juice ion exchange softening positively affects downstream operations. It eliminates the need for descaling evaporators and improves efficiency of heat transfer. The softening process also improves boiling characteristics of juice and has a positive effect on sugar quality. Cane juice typically has higher hardness than beet thin juice, which means that larger softening systems could be required and more regenerant will be produced. Also the presence of higher suspended solids in cane juice makes it difficult to use fine ion exchange resins. It has been proven that softening of membrane filtered clarified juice is quite efficient. However, the cost of membrane filtration remains relatively high. A fractal softener with a short bed has been tested for unfiltered clarified juice. Test results and the benefits of clarified juice softening are discussed.

INTRODUCTION

Softening of sugar juices and molasses has become an established unit operation in the beet sugar industry, especially in factories using chromatographic technology for molasses desugaring. Because of the negative effect of divalent cations on separation characteristics of chromatographic resin, juice or molasses softening is a necessary part of the process. It has been noted that processing soft juices and syrups positively affects the downstream operations. It eliminates the need for descaling evaporators and improves heat transfer efficiency. The softening process also improves boiling characteristics of juice and has a positive effect on sugar quality. Several ion exchange processes have been developed and implemented over the years.

Reviews of these methods can be found elsewhere (Dorfner, 1991; van der Poel, 1998). The cane industry so far has not taken advantage of the softening process.

Although much attention has been paid by various researchers to resin regeneration and regenerant waste handling, relatively little has been done for development of more efficient equipment. Conventional ion exchange comprises regular tanks with relatively simple fluid distribution systems. Typically larger diameter tanks are less efficient because of fluid maldistribution. These inefficiencies are often remedied by increasing resin bed depth, which leads to underutilization of ion exchange capacity, excessive regenerant use and unnecessary dilution of process streams. More advanced systems, such as Calgon Carbon's ISEP (Snyder, 1999) use countercurrent carousel design, allowing exhausting resin beds more completely. They also use smaller process vessels that reduce problems with fluid distribution.

New opportunities for design of ion exchange equipment arise with the implementation of engineered fractals for fluid distributors (Kearney, 2000; Kochergin, 2001). By providing nearly ideal fluid distribution, fractals allow utilization of very shallow resin bed and therefore, significantly reduce required amount of resin. Kearney (2001) has presented data that demonstrated advantages of using fractal distributors for softening of beet thin juice. Table 1 illustrates the differences in softening systems utilizing conventional and fractal softeners. The new equipment is about ten times smaller compared to conventional systems due to more efficient distribution and the possibility of faster cycling. Smaller pressure drop allows building cheaper equipment and saving energy.

Table 1. Comparison of conventional weak cation juice softening system with an industrial flatbed fractal device (Amalgamated Sugar Company, Paul factory, Idaho, USA)

Parameter	Conventional (lateral orifice distributor)	Fractal Flat Bed
Resin bed depth, m	1.0	0.15
Exhaustion flow rate, BV/hr	50	500
Bed pressure drop, bar	3.5-5.6	0.1
Regeneration flowrate, BV/hr	30	150

Softening in cane mills has not been considered as a standalone operation, mainly because of the presence of suspended solids in clarified juice. Because of the possibility of plugging the resin bed with suspended solids, clarified juice has to be filtered prior to ion exchange. Suspended solids level in beet thin juice (about 5 ppm) is much lower compared to cane clarified juice (up to 200 ppm). Higher suspended solids level in cane juice makes it difficult to use fine ion exchange resins because of the danger of plugging. Cane juice typically also has higher hardness compared to beet thin juice, which means that larger softening systems could be required and more regenerant will be produced. Therefore, softening of clarified juice is typically considered in combination with other separation methods developed, for example, for white sugar production directly in the cane mills, (Kochergin, et al., 2000; Fechter, et al., 2001). It has been proven in many publications that micro- or ultrafiltration provides a juice of sufficient quality to avoid plugging of ion-exchange beds. Although membrane filtration positively affects cane mill operation, the cost of membrane systems is still relatively high to justify the required investment.

If the softening process could be carried out without prior membrane filtration and the overall softener size and cost could be reduced, the process would be of interest to cane processors worldwide. A fractal softener with a short bed has been tested for filtered and unfiltered clarified juice. Test results and the benefits of clarified juice softening are discussed below.

EXPERIMENTAL SECTION

Tests have been performed with membrane filtered and unfiltered cane clarified juice. A pilot fractal softener (3.24 sq. ft. cross section, resin bed height – 6- 10 inches) was installed at the Sugar Cane Growers Cooperative of Florida mill (see figure 1). Clarified juice was prescreened through a Filtomat screen with a 100 micrometers slot size. The first set of tests was carried out using clarified juice filtered through a Koch UF membrane (MWCO around 100,000). A DOWEX strong cation resin in Na⁺ form was used for softening. Flowrate was maintained at about 55-60 BV/hour. Resin regeneration was performed using NaCl solution –1.78 eq/l Na – up to 180 % on operating capacity to fully regenerate resin. Over a hundred exhaustion - regeneration cycles were performed. Average feed brix- 11.7%, feed limesalts (determined by EDTA titration) - about 22.5 meq/100g DS. Average service cycle was about 70 minutes. Typical steps involved in a full production cycle are listed in Table 2. The sequence of steps and flow direction may vary depending on the selected process configuration.

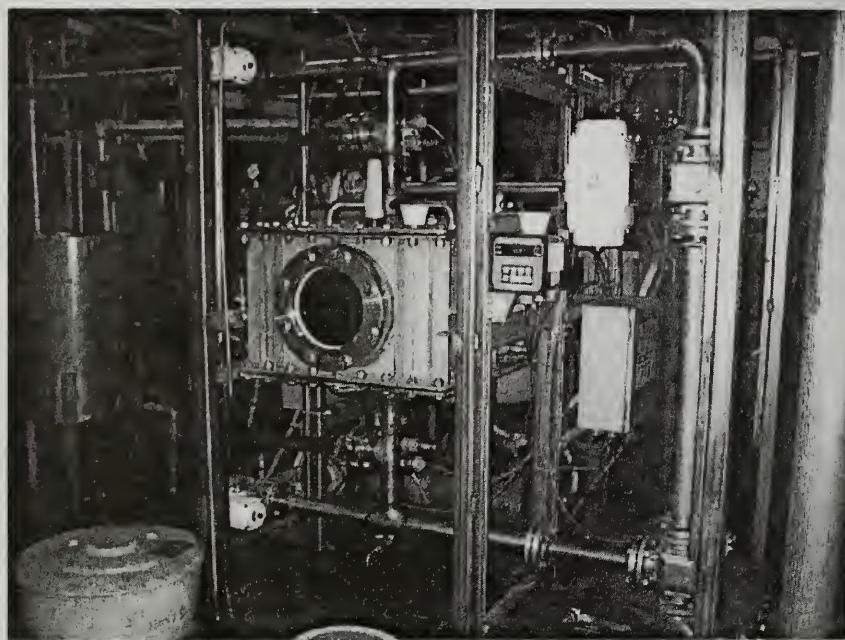


Figure 1. Pilot Fractal softener

Nine tests were run using unfiltered clarified juice to observe if the softener could operate without membrane filtration. The bed height was reduced to 6 inches to allow full resin expansion during the backwash stage. Juice flowrate was reduced accordingly. Pressure drop was monitored across the resin bed as well as the top and bottom fractals to determine if any irreversible plugging took place. Juice hardness was analyzed periodically by EDTA titration method to determine the breakthrough point.

Table 2. Typical softening steps

Step	Effluent stream composition	Comments (where to send the stream)
Sweeten on	Displaced water	To sweet water tank
Exhaustion (softening)	Soft juice	To mill evaporators
Sweeten-off	Dilute soft juice	To softener supply tank or mill evaporators
Resin backwash	Sweet water and suspended solids	To drum filters
Regeneration	Mixed chlorides (for strong acid in Na-form) Acidic Ca, Mg sulfate waste (for weak H+form)	Waste stream
Regenerator rinse	Dilute regenerator	Regenerator supply tank

RESULTS AND DISCUSSION

The results for seven tests with unfiltered clarified juice are summarized in Figures 2 through Figure 4. Increase in pressure drop was plotted against time of the softening cycle for top and bottom fractals and resin, respectively. Increase in pressure drop gives indications of plugging of fractal channels or resin bed with solids. Figure 2 illustrates that pressure drop increase was relatively small during the cycle in all but one case. Higher-pressure drop increase in that case was related to disruption in clarifier performance, which led to increased level of suspended solids in the feed material. The same trend can be observed for the resin bed, where pressure drop increase in test 4 was sharper than in the other tests. However, the pressure drop in the bottom fractal did not follow the same trend, indicating that the solids must have been held by the resin and did not have time to reach the bottom fractal within one operating cycle. Overall, the values for pressure drop in the bottom fractal did not exceed 0.35 psi. The fact that pressure drop returned to the original value every time after backwash indicates that no irreversible resin or fractal fouling took place.

It is important to mention that the operating capacity of 1.7-1.8 eq/l was reached consistently throughout the tests. The same resin capacity was reached with membrane-filtered juice. The key conclusion is that a certain accumulation of suspended solids in the resin bed did not interfere with the softening process. Figure 5 shows a typical softening breakthrough curve for unfiltered juice also showing certain pressure drop increase during the operation cycle.

After the end of the test program it was discovered that the resin still contained suspended particles, which was due to insufficient backwash pump capacity.

Figure 2 - Unfiltered Clarified Juice Tests
Top Fractal

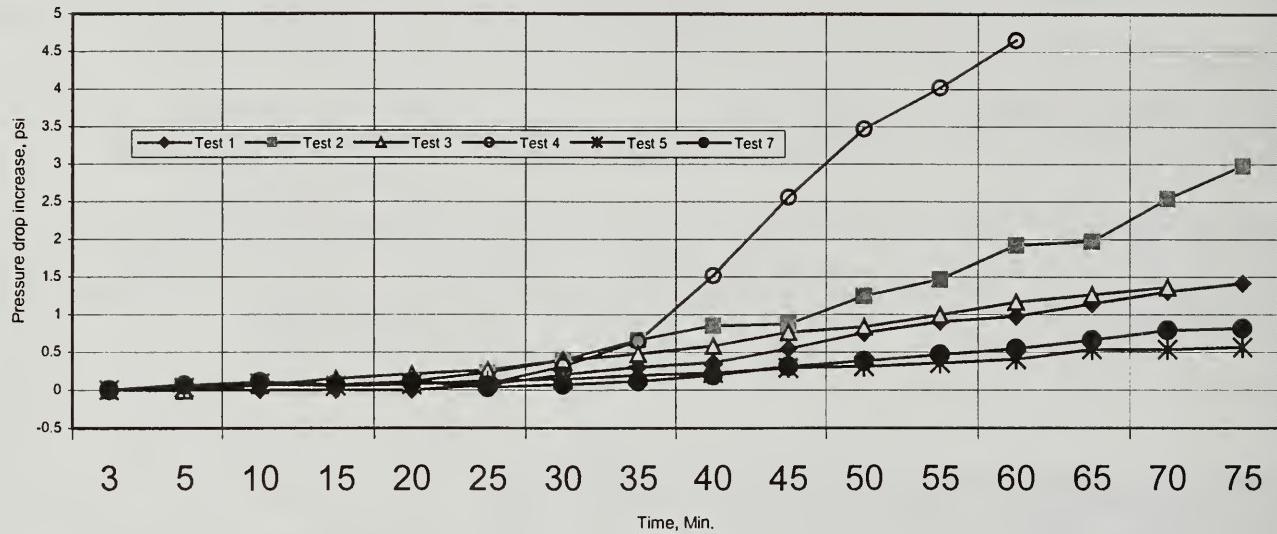


Figure 3-Unfiltered Clarified Juice Tests
Bottom Fractal

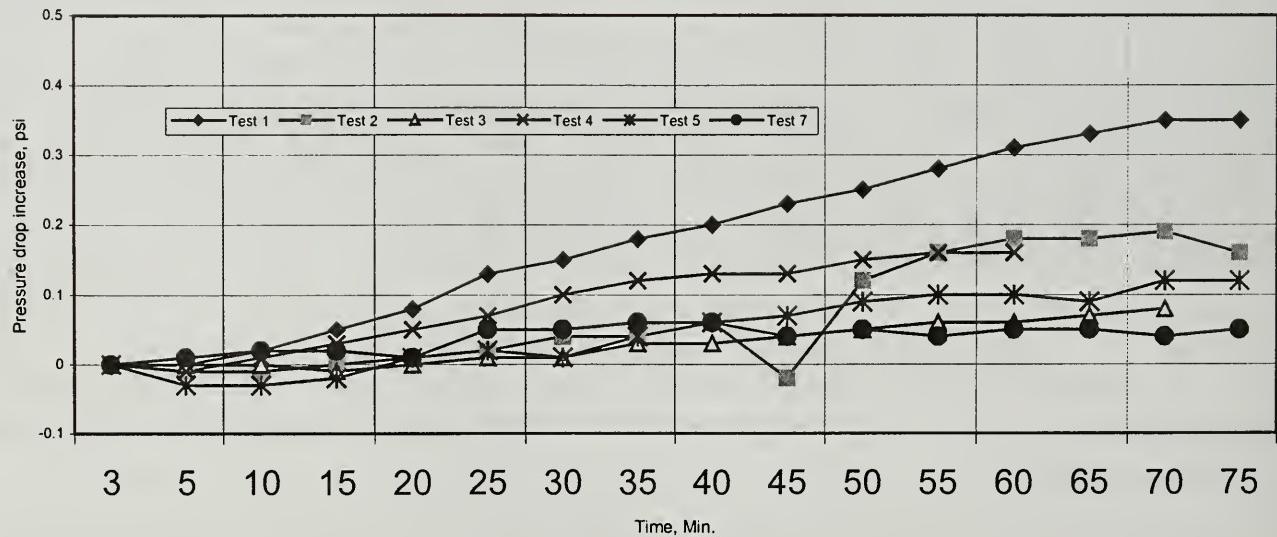


Figure 4- Unfiltered Clarified Juice Tests
Resin pressure drop

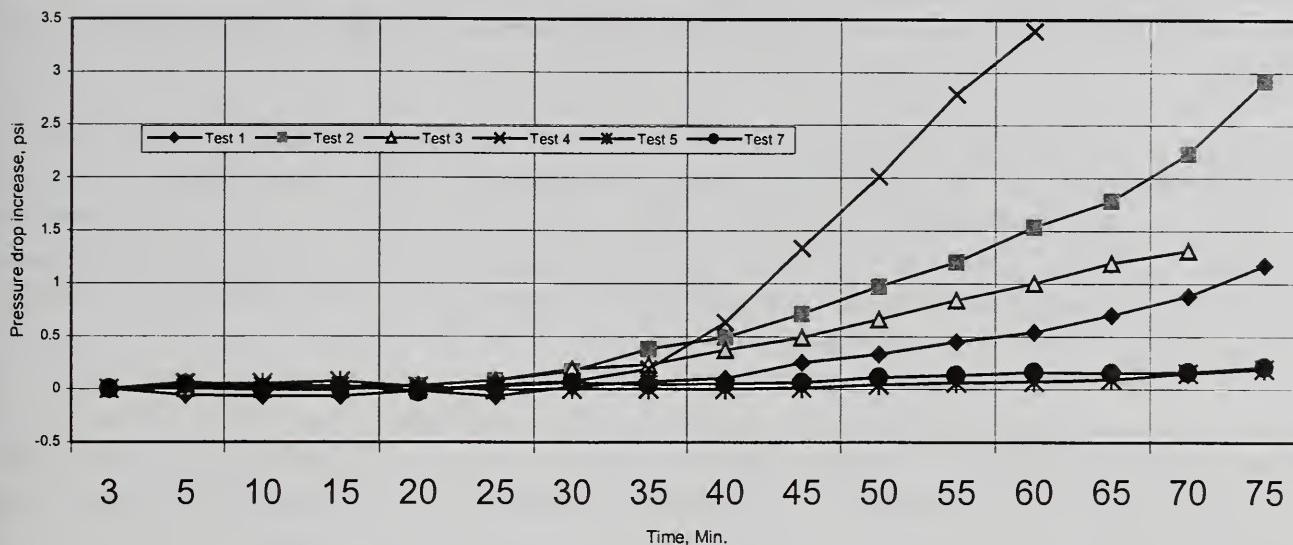
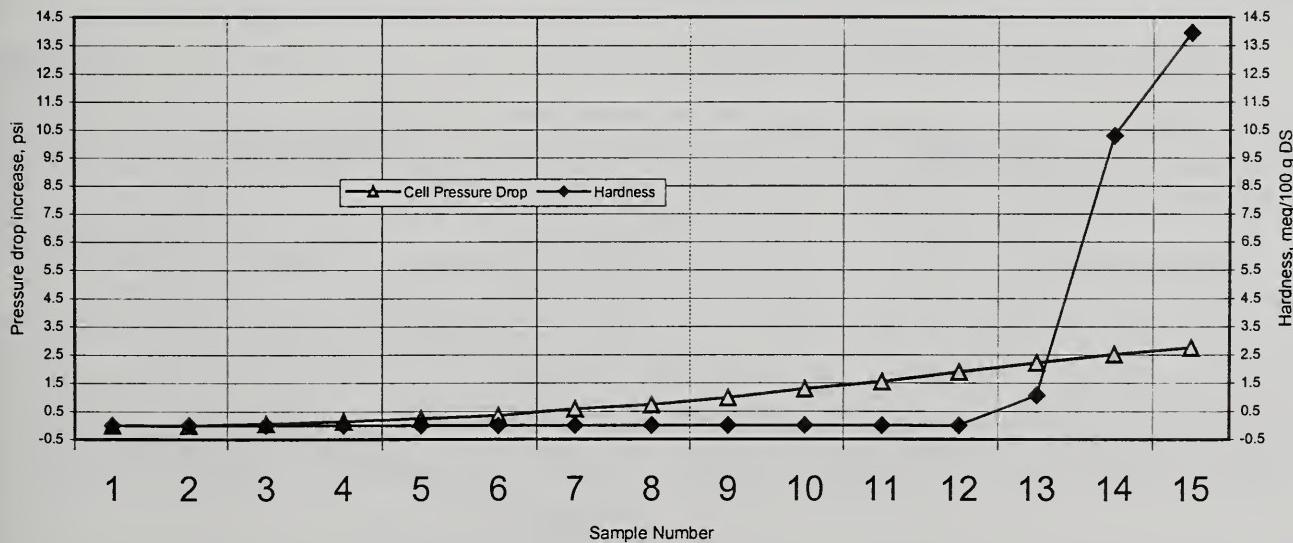


Figure 5 Unfiltered Clarified Juice Tests
Typical breakthrough curve



Benefits Of Clarified Juice Softening

A short summary of expected benefits if clarified juice softening is implemented in a cane mill juice is shown in Table 3. The following discussion explains the potential advantages of juice softening.

Experience with beet sugar factories employing the juice softening technology indicates that no evaporator boilouts are required. This results in savings in chemicals, labor, repair and maintenance costs associated with boilouts. Because fully softened juice may become corrosive, a decision has to be made about the required level of softening. The residual concentration of calcium in the juice can be adjusted by extending the softening cycle and if necessary allowing a certain amount of calcium to leak through the softener.

Gradual evaporator scaling during the period between the boilouts reduces overall heat transfer efficiency. In the case of softened juice the heat transfer coefficients stay constant, thus maximizing evaporator performance. In addition, the evaporator capacity unused during boilouts becomes available, when boilouts are eliminated. It may become an additional benefit for the factories where evaporation creates a bottleneck for capacity increase.

Potential increase in raw sugar quality deserves a separate discussion. The benefits, however, are hard to substantiate without running a full-scale test program. Clearly, elimination of calcium ions that tend to form insoluble salts should benefit the crystallization process downstream and improve the quality of final product. At the same time, elimination of scale on the heating surface in evaporators and vacuum-pans will possibly allow reducing the temperature of heating steam and potentially alleviate color buildup. These benefits cannot be easily proven on the pilot-scale. Therefore, large-scale tests are recommended.

Table 3. Softening benefits summary

Benefits	Comments
Eliminate evaporator boilout	No calcium salts in the solution
Reduce chemical cost	Some chemicals will still be used for softening regeneration
Energy savings	See the discussion above
Labor and repair savings	
Potential for capacity increase	Availability of additional evaporation area
Potential improvement in raw sugar quality	Difficult to substantiate, needs to be tested on large scale
Potential color reduction	Lower temperature difference

Strong Cation Vs. Weak Cation Softening

Weak cation resins typically have higher operating capacity compared to strong cation resin (3.0-3.5 eq/l resin for cane juice), which would result in smaller sized equipment. In the beet industry weak cation resins are used in H⁺ form. Regeneration is performed with dilute sulfuric acid. It had been proven for many years of industrial operation that the risk of sucrose inversion is negligible.

In the case of weak cation resins, regenerant is used in excess of 10-20% over stoichiometric requirements. The main characteristic of weak cation softening is the production of large amounts of dilute regenerant. Acid dilution is required to prevent the formation of insoluble calcium salts (gypsum) in the resin matrix. In the beet industry thin juice softening is beneficial, because the waste liquid is used as a pressing aid for beet pulp (Henscheid, 1990). With a relatively low level of initial thin juice hardness (after carbonation) the total amount of softening regenerant is used in the process. There is no information in the literature, if addition of gypsum provides any advantages for cane milling. Also, with cane juice hardness being 2-3 times higher than that of the beet juice the total amount of dilute calcium sulfate solution will be proportionally higher.

Strong acid softening resins are typically used in Na-form and regenerated with about 2N brine solution. As a result, regenerant is a rather concentrated solution of calcium, magnesium and sodium chlorides. The total operating exchange capacity of strong resin for cane juice reaches 1.7-1.8 eq/l of resin. The amount of regenerant typically exceeds the stoichiometric requirements by 50-80 %. The large amount of sodium in the waste stream may create an environmental issue for some locations. ARI has developed and tested on a bench scale a method allowing reduction in total waste by a factor of 10-15, which makes the application feasible. Another advantage of strong cation resin over weak cation resin for cane applications is its temperature stability, since clarified juice temperature is close to boiling point.

System Size

Conventional softening systems are characterized by relatively large size and high resin bed, making them essentially unsuitable for softening of unfiltered cane clarified juice. For example, a softening installation was described for processing of about 1000 gpm of membrane-filtered cane clarified juice, consisting of two columns, each containing 45 m³ of strong cation exchange resin and 15 m³ of inert materials (Kwok, 1996). The inert materials must be used to shield the liquid distributors from the resin. Each column is 7.6 m high and 4 m in diameter. Softeners had to be regenerated every 8-10 hours: 80 % hardness removal has been reported. It is obvious that the described installation would not be able to perform on unfiltered clarified juice. Because of the presence of suspended solids such installation can turn itself into a depth filter because of the long cycles and bed height.

Our estimates show that for fractal installations characterized by a short bed and fast cycling only about 3.4 m³ for each unit would be necessary. No inert material is required. The accumulated solids will be removed during backwash cycle as was demonstrated in our tests. Short cycles mean that less solids will be “filtered” through the bed between backwash cycles. Shallow bed depth will assure the depth filtration is minimized and more fine solids are passed through without being held inside the bed.

CONCLUSIONS

1. The softening process can be accomplished on both filtered and unfiltered clarified juice. In the short bed fractal systems, accumulation of suspended solids in the resin bed during the operation cycle did not result in reduction of resin capacity. Solids buildup is dependent on total concentration of suspended solids in the juice. A reliable prescreening will be essential to prevent solids from building up in the resin bed or distributors.
2. Use of fractal distributors in the softeners allows accomplishing the softening process using a shallow bed with a small amount of resin. Frequent cycling and backwashing allows avoiding significant buildup of solids in the resin bed. Pressure drop across the softening cells did not exceed 4 psi over the length of the operating cycle. Cell construction allows "out of cell" backwashing if it becomes necessary.
3. A decision on the type of softening resin depends on the environmental regulations and process configuration of a particular plant. A method of regenerant reuse for strong cation resin has been developed.
4. Longer large scale testing is recommended to verify that the softening system can withstand normal process fluctuations.

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DEVELOPMENT OF AN ONLINE NIR SYSTEM FOR THE ANALYSIS OF QUALITY PARAMETERS IN INDIVIDUAL CONSIGNMENTS OF SHREDDED CANE

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ABSTRACT

In South Africa (SA), individual consignments of shredded cane are tracked electronically and analysed by the direct analysis of cane method (DAC). Although this procedure is capable of producing excellent sub-sampling and accurate analysis of consignments, it is labour intensive. NIRS has been used by several sugar industries for determining cane quality for cane payment purposes. This paper summarises the progress of NIRS, in the South African Sugar Industry, from an atline test rig to a fully automated online operation.

INTRODUCTION

Cane tracking, sub-sampling and quality analysis

The Cane Testing Service (CTS) in South Africa is responsible for demarcation, tracking and analysing all consignments of incoming cane to determine the distribution of financial proceeds between growers. Cane is analysed using the direct analysis of cane method (Buchanan and Brokensha (1974)). When a new consignment passes the entry point on the first conveyor, the cane tracker attendant presses the start button, the consignment is tracked electronically through the knives and shredders on its way to the DAC sub-sampling station. When the consignment arrives, a sampling hatch at the head of the conveyor leading to the diffuser (or first mill chute) is enabled. The duration of a single sampling cut is approximately 0.7 seconds with an average size of 60 kg. A twenty ton consignment will typically produce some 6 cuts (final sample size of 360 kg) which discharges into a continuous 2-stage reciprocating sub-sampler (Figure 1). Final sample size for despatch to the CTS laboratory is 5 kg. Buchanan and Brokensha (1974), using both static and dynamic tests, have shown that this sampling system is free from bias and sampling precision was calculated at 0.3 units of pol % cane. Laboratory tests of cane quality parameters of individual consignments include pol, brix, moisture, fibre and ash. Although it is accepted that this procedure is capable of producing excellent sub-sampling and accurate analysis of consignments, it is relatively slow, labour intensive and expensive.

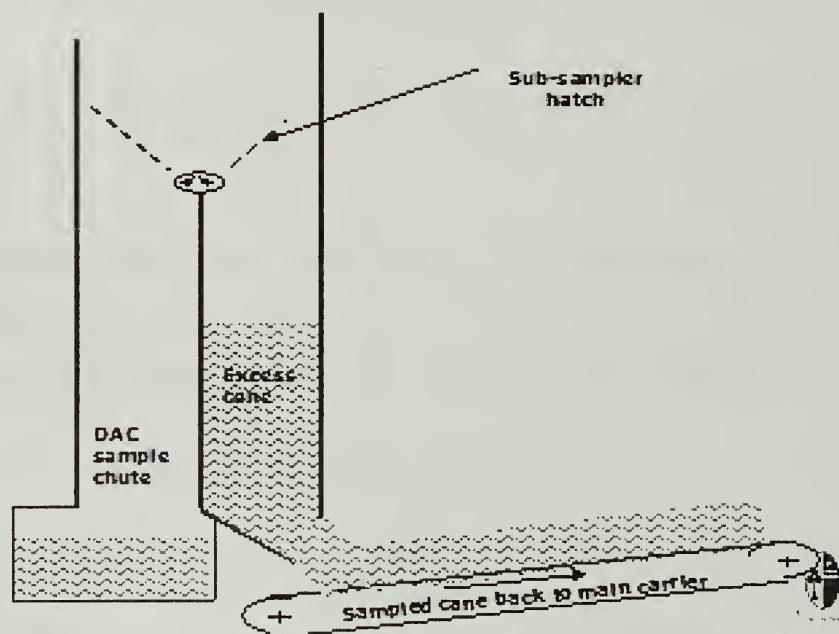


Figure 1. Schematic of DAC sub-sampler showing reciprocating sub-sampler, DAC sample chute and excess cane returning to process

Use of NIRS for shredded cane testing

Laboratory and atline NIRS has been proposed for shredded cane analysis by Brotherton and Berding (1995), Edye and Clarke (1996), Schäffler and Meyer (1996) and Peterson and da Silva (1998). All these studies have shown that a standard error of prediction (SEP) ranging from 0.4 to 0.6 for brix with little bias can be obtained.

However NIRS is expensive and in South Africa laboratory-based NIRS cannot provide significant additional benefits to be financially viable. Conventional laboratory procedures routinely determine pol, brix, moisture, fibre and total ash in consignments. NIRS can only be justified if the procedure can save money, this in turn means that NIRS needs to be applied online.

Researchers at the Bureau of Sugar Experiment Station in Australia have led the way with online NIRS. Their CAS system has been commercialised and is used routinely in several Australian and Fijian raw sugar factories (Staunton *et al*, 1999, Watson *et al*; 1999 and Habib *et al*, 2001).

Electronic cane tracking and the DAC sub-sampling method, used in SA, provide a unique application of NIRS. This is due to:

- the existence of a system for identification, demarcation and monitoring of each consignment
 - the availability of an efficient sub-sampling system for individual growers' consignments
 - the fact that analysis of the sub-samples of these consignments is routinely available.
- Shredded cane and not juice is analysed so fibre as well as other quality components are available. Implementation of online NIRS at the DAC station is much simpler than sampling and analysing cane on the main carrier.

Atline testing of an Online NIRS instrument

Initial testing of a NIRSystems Direct-Light process NIRS was carried out atline in the Maidstone mill CTS laboratory in 2000, a special test rig was developed, see Figure 2. The post-sample dispersive Foss instrument allows for the online analysis of analytes on conveyor belts and other process applications. Over 1200 samples were used, calibration and prediction sets were independent of each other (Schäffler, 2001). Prediction results are shown in Table 1.



Figure 2. Atline test rig for evaluating Foss 6500 direct light NIRS process analyser

Table 1. Prediction results, calibration set from Aug-Oct 2000, prediction set from Nov-Dec 2000

Analyte	SEP	Bias	Slope	RSQ
Pol	0.4	0.1	0.94	0.92
Brix	0.4	0.1	0.90	0.84
Moisture	1.0	-0.2	0.94	0.76
Fibre	1.1	0.3	0.91	0.69
RSQ = correlation coefficient squared				

The results, using the post-sample dispersive NIRS system were very similar to those published by others using a pre-sample dispersive grating spectrophotometer.

INDUSTRIAL APPLICATION

Phase 1 - Development of Online semi-automated NIRS

Description of system. As DAC sampling stations already have two vertical chutes for manual sampling it was logical to add an additional chute for NIRS scanning. The NIRS chute incorporates a set of metering mills to ensure cane build-up in the chute. A glass window just above the rolls allows light from the NIRS's scanhead to interact with the moving cane. A capacitative level sensor (Sensorik 50/10) was installed about 1.5 meters above the rolls. This sensor activates a reciprocating third hatch ensuring that a constant head of cane is always in the chute. A schematic of the NIRS sampling system is shown in Figure 3.

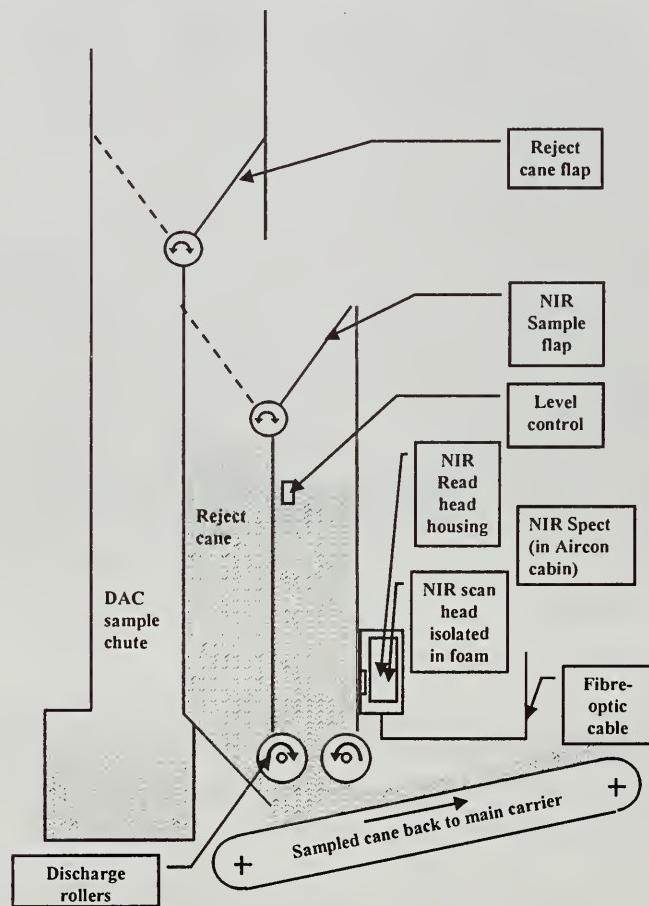


Figure 3. Schematic of DAC station with NIRS chute, readhead, rolls and level sensor

A small fibreglass air-conditioned cabin was installed next to the sampling station to house the Foss NIRSystems 6500 process spectrophotometer. The instrument was mounted on one of the walls using anti-vibration mountings allowing the scanhead, on a 2 meter fibre-optic cable, to be mounted adjacent to the sampling chute. The cabin also contained a control PC, control logic for operating the rolls, compressed air lines for running the NIRS reference and cooling the scanhead (Figure 4).

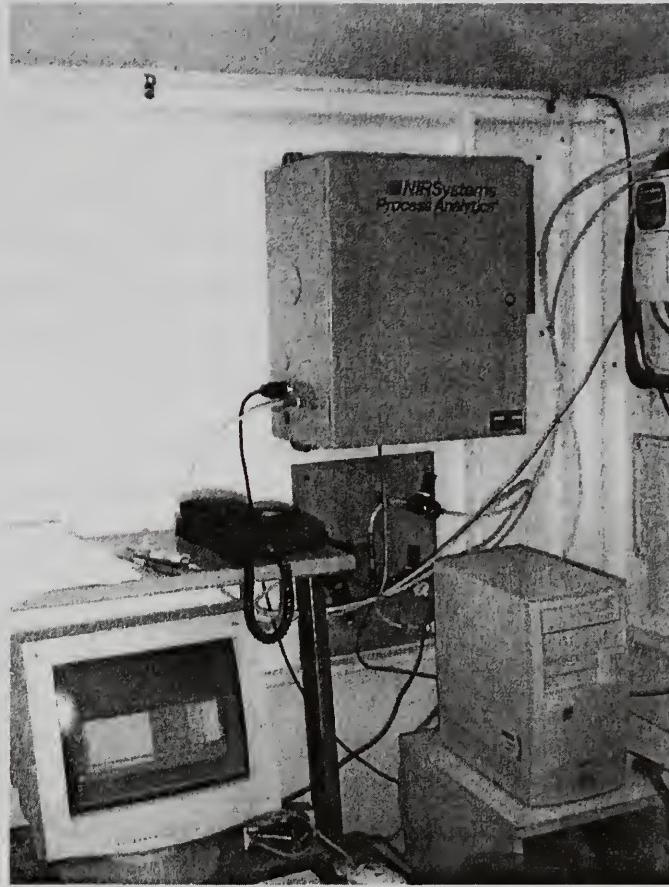


Figure 4. Internal view of the NIRS cabin showing online NIRS and additional equipment

Foss recommend that the effects of factory vibrations on the NIRS instrument be reduced through vibration damping. This was tackled in two ways:

- Air springs, from Firestone Industrial products, were used to provide isolation from factory vibrations. A two part steel frame, supporting the cabin, was constructed with an air spring mounted in each corner. These were maintained at 250 kPa.
- The scanhead was housed in a glass-fibre box and was surrounded by soft foam to reduce the effects of factory vibrations. A special mounting bracket ensured that, after routine maintenance, refitting was reproducible. The unit was sealed against the chute, using anti-vibration foam, and positive air pressure was applied to prevent dirt or moisture ingress. As an additional safety precaution a metal screen surrounded the box.

Setting up the DAC sampling station prior to NIRS scanning. Maidstone has two diffusers. Throughput ranges from 170 to 220 tons cane per hour for each diffuser. Not all consignments are analysed and some four to five DAC analyses are carried out per hour for each diffuser. The size of the consignments varies from 8 to 25 tons. The amount of cane moving through the station under manual sampling conditions is relatively small. For online NIRS scanning, higher throughput is necessary. Prior to scanning, the NIRS tester alters the timers, increasing the frequency of the main hatch opening, sending more cane to the station. In addition the ratio of cane sent to the NIRS chute relative to the manual sampling chute is also increased.

Collection of spectra. WINScan v1.04 from Foss NIRSystems was initially used for all NIRS scanning. Although the NIRS was online, scanning was done manually. A bell in the NIRS cabin would indicate that a cane consignment had arrived at the DAC sampling station. A monitor in the cabin graphically indicated tracker number and the progress of each consignment. The consignment number was manually entered into WINISI. A scan takes approximately one minute (50 seconds for the sample and 10 seconds for the reference). This process was repeated until the bell signalled the end of the consignment. If the final sub-sample was still being scanned when the consignment ended it was included for subsequent averaging as the sample (due to the lag-time) would still be in the NIRS chute. On average, four scans were obtained per consignment.

Melding spectra to laboratory data and calibration development. At the end of each day, the tester retrieved pol, brix, moisture, fibre and ash data from the CTS laboratory for each consignment and manually merged this data with the relevant spectra. A procedure for uploading spectra and CTS laboratory data to the SMRI was developed using modems and PCAnywhere software (v8.01). This semi-automated system ran virtually trouble-free from July 2002 to the end of the season (mid-December 2002). As the online procedure was only semi-automated, samples were only collected during the day shift. Spectra were scanned from 880 to 2300 nm. Sub-samples of each consignment were averaged (WINISI v1.04). The calibration set consisted of 650 consignments (from a total of 2225 scans) collected from 25th July to 15th October 2002. Spectral outliers were removed (Global Mahalanobis distance (GH) > 3). Typical variation for each analyte in the calibration set is shown in Table 2.

Table 2. Cane quality parameters in shredded cane: NIRS calibration dataset (n = 650 collected from July to October 2002).

Analyte	Mean	Min	Max	SD
Pol	12.6	9.6	16.1	1.0
Brix	14.8	12.3	18.2	0.9
Moisture	69.9	63.9	75.3	1.9
Fibre	15.6	11.6	21.4	1.8
Ash	1.2	0.3	7.0	0.7

Calibration equations were developed, using WINISI's PLS process. The calibration statistics were compared to those from tests carried out in 2000 under "ideal conditions" (offline in an air-conditioned laboratory, cane packed manually into a sample compartment, no vibration problems), see Table 3.

The calibration results from the two tests were remarkably similar, indicating that the transition strategy from laboratory to online was sound.

Table 3. NIRS calibration statistics for cane quality parameters in shredded cane: Comparing Inlab and Online calibration results.

Analyte	Laboratory trial		Online trial	
	SEC	RSQ	SEC	RSQ
Pol	0.3	0.91	0.4	0.86
Brix	0.2	0.94	0.3	0.88
Moisture	0.8	0.86	0.7	0.85
Fibre	0.9	0.8	0.8	0.80
Ash	0.2	0.5	0.4	0.40

Validation of NIRS equations. It is important when testing NIRS equations that the calibration and validation sets be independent of each other. For this reason the test set consisted of samples scanned from 21th October to 29th November 2002. Figure 5 shows the distribution of the validation samples as a distance from the mean of the calibration score file. The mean GH value was 1.1 indicating that the prediction file was well within the limits of the calibration library. The predictions of the validation set are shown in Table 4.

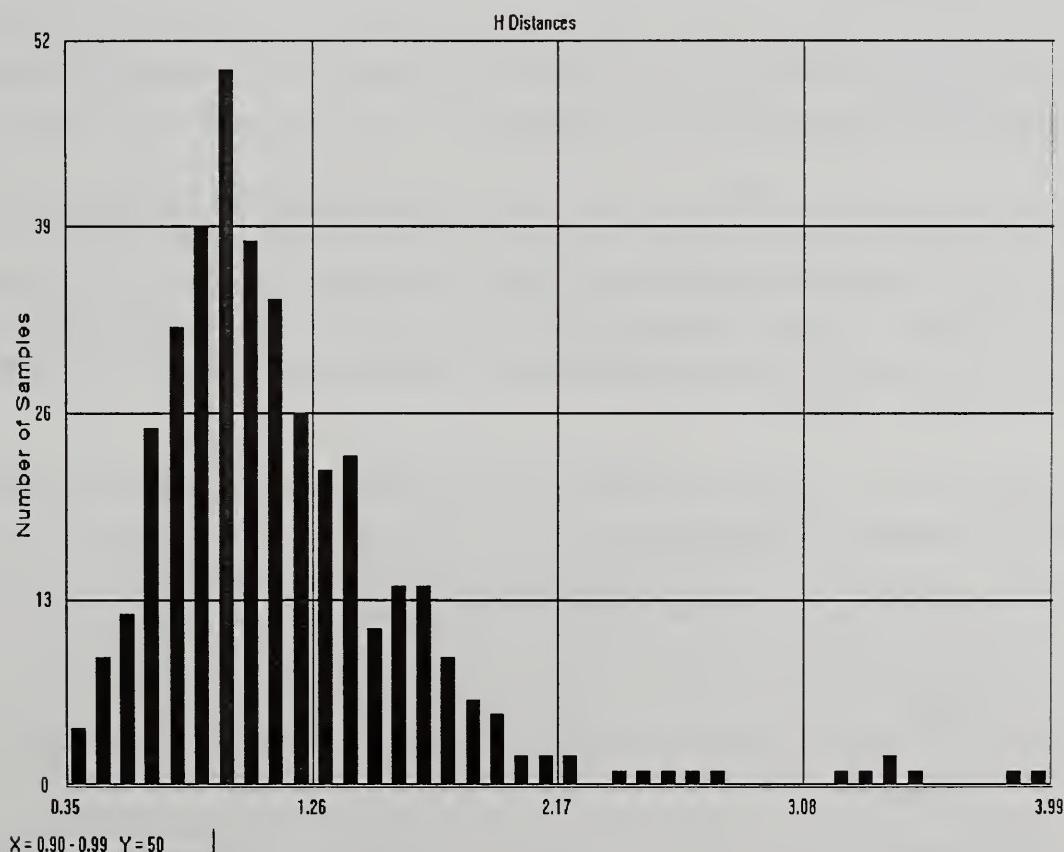
**Figure 5.** Histogram of Global Mahalanobis distances (GH) for the prediction set samples from the calibration mean, indicating that the two sets are similar

Table 4. Online NIRS prediction results for analytes in 371 consignments of shredded cane

Analyte	SEP	Bias	Slope	RSQ
Pol	0.5	-0.3	0.84	0.72
Brix	0.4	-0.1	0.90	0.74
Moisture	0.9	0.3	1.03	0.76
Fibre	0.9	-0.1	0.92	0.67
Ash	0.6	0	1.23	0.26

The standard error of prediction (SEP) is a measure of the scatter between laboratory and NIRS predictions. Bias is a measure of NIRS accuracy, it is the mean difference between laboratory and NIRS results. The results in Table 4 show that:

- for online predictions, pol and brix results were very good, scatter and bias were less than half a unit. Brokensha and Mellet (1977) established that errors of 0.4 units of pol % cane could be expected from DAC sampling and analysis.
- a SEP of less than one unit for moisture is most satisfactory. On average this is a relative error of less than 2%.
- an NIRS SEP of 1 in 15 for fibre is therefore reasonable since fibre % cane is derived from moisture and brix % cane. In practice when using NIRS, it is preferable to calculate fibre from the NIRS estimates of brix and moisture.

Another way of evaluating the NIRS prediction results is to compare the NIRS precision (SEP) to the precision of the conventional laboratory methods. The precision of the DAC procedure was recently investigated by Schäffler and de Gaye (2002). The summarised results are included in Table 5. It is obvious from these results that the NIRS SEP are similar to the standard deviation (SD) of sub-sample replicates for all analytes, reinforcing the findings that Online NIRS predictions are suitable for routine cane analysis.

Table 5. Comparing the precision of NIRS predictions with results obtained by conventional analysis (4 CTS laboratories, 274 samples)

Analyte	Laboratory SD	NIRS SEP
Pol	0.3	0.5
Brix	0.3	0.4
Moisture	1.1	0.9
Fibre	1.5	0.9
Ash	0.4	0.6

Ash predictions. Total ash estimates are extremely poor, see Table 4. These results are similar to those previously published (Schäffler, 2001). In 1997, Schäffler and de Gaye found that although NIRS ash results for molasses (high ash content) were excellent, the same could not be said for mixed juice (low ash content, RSQ = 0.55). This is in contrast to reports by other investigators. Madsen *et al* (2002), using a Foss Direct Light NIRS in a batch mode, found that a logarithmic function produced a reasonably strong correlation, however the sand (dirt or mud) levels in Louisiana were extremely high (average ash = 5% with levels peaking at 10 to 30%). In the current study an equation was generated using log ash as a new analyte. The prediction was then converted using antilogs; the results did not show any improvements to those summarized in Table 5. Staunton *et al* (1999) found reasonable ash predictions (SEP = 0.5, RSQ = 0.71 with a slope of 1.00). A slope of unity is in contrast to that found by Madsen *et al* (2002) and this work.

The poor ash predictions in South African DAC samples are probably due to the:

- low absorbance of ash as inorganic substances have little absorbance in the NIRS
- low total ash levels in SA cane (0.5 - 1.5%). NIRS is not a sensitive technique. In the prediction set 90% of the samples had ash levels less than 2%
- precision of the laboratory ash technique. Brokensha and Mellet (1977) found that, under ideal conditions, ash precision was ± 0.3 units. Assuming ash in cane was 2%, this relates to a relative error of 16%. In routine CTS laboratories greater errors can be expected. As NIRS calibrations are dependent on this laboratory procedure, the NIRS estimates will include this scatter.

Ash predictions in SA DAC samples are more likely to be qualitative than quantitative. However selection of samples for a future database needs to be carefully selected in order to expand the range.

Phase 2 - Development of OnLine NIRS

Online scanning of shredded cane at the MS station was limited to a semi-automatic operation. An operator manually entered each consignment number and started the NIRS manually. The operator also had to decide how many sub-scans to do for each consignment. Steps to automate the procedure included:

- upgrading the NIRS scanning software from WinISI to ISIScan v1.26. Using a separate NIRS control program (NIRS-CP, produced in-house at the SMRI), each consignment number together with other command strings is sent to ISIScan via a LIMS import folder. Likewise once ISIScan has scanned a sub-sample, the NIRS results are sent via a LIMS output folder to the NIRS-CP. A script for the factory's LIMS system (Sample Manager) was written to accept NIRS predictions for easy comparison with the conventional laboratory data.
- modifying the CTS cane tracker software to send consignment start/stop signals to the NIRS-CP via a serial link. The consignment number is also transmitted at this time.

Moving from manual to automated scanning demanded that the control system could:

- identify new consignments
- scan multiple sub-samples of each consignment

- cope with cane carrier stops
- manage changing carrier speeds
- ensure that each NIRS sub-sample scan is valid (*i.e.* ISIScan must only scan if there is enough cane in the chute for a 50 second scan).

These objectives were addressed by:

- adding two additional cane level sensors to the NIRS chute
- developing an interface between the NIRS-CP and the hardware
- ensuring that NIRS-CP enabled the opening and closing of the sample hatch on the NIRS chute
- ensuring that discharge rolls were also under the control of NIRS-CP.

Description of automated NIRS scanning. Before the arrival of a new consignment the NIRS hatch is disabled, *i.e.* no cane is allowed down the NIRS chute. The motors are also disabled. This ensures that when a new consignment arrives the chute will fill-up rapidly. When a new sample arrives (new consignment number) the sampling hatch is enabled. The 3 level sensors are shown on the NIRS-CP computer screen, see Figure 6. As the chute begins to fill up with cane the “chute-empty” sensor will activate, however the motors will remain off and only when the “cane-ready” sensor is activated will the NIRS begin scanning and the motors come on. Eventually the “chute-full” sensor will activate and keep cane in the chute at this level. Sub-samples are scanned continuously until the NIR control program receives an end-of-consignment signal, the sampling hatch is then disabled. Even then scanning continues until the “cane-ready” sensor is de-activated as cane drops below this level. The motors remain on until the “chute-empty” sensor is deactivated purging all remaining cane from the chute, the system is ready for the next consignment. If the carrier stops or slows down, during the sampling of a consignment, the cane ready sensor will deactivate. Under current conditions (motor speed of 30 Hertz) and program logic, there is enough cane in the chute for a 50 second scan. If cane drops below this level, the system will wait until the carrier restarts and the “cane-ready” sensor is re-activated.

Testing the automated NIRS-CP/ISIScan system. Fully automated at-site tests at Maidstone sugar mill ran successfully from 27th October 2003 until the mill closed on 14th November 2003. This included unattended overnight scanning. A total of 830 scans from 171 consignments were carried out. Advantages of the fully automated system were:

- improved quality of the scans. Out of the 830 scans only 5 scans produced Global Mahalanobis values (GH) that were greater than 3 and these ranged from only 3.1 to 5.2. With the manual online approach many scans were invalid due the chute not always being completely full and the resultant voids producing anomalous scans.
- many more scans. Over a twenty four hour period the online system produced 336 scans. It is here that NIRS can be cost-effective as the NIRS attendant and the DAC sampler operator are not required.

Potential savings of the automated NIRS system. The potential savings of the NIRS system as a replacement for DAC can be evaluated by comparing NIRS to DAC costs. In these difficult times of low world sugar prices, a weak US dollar, low local prices and drought problems, sugar

economists are demanding pay-back periods of less than 2 years. Although it is difficult to provide accurate economic information, NIRS does appear to offer an attractive alternative to the current manual DAC system, with a payback period of about 16 months for local conditions.

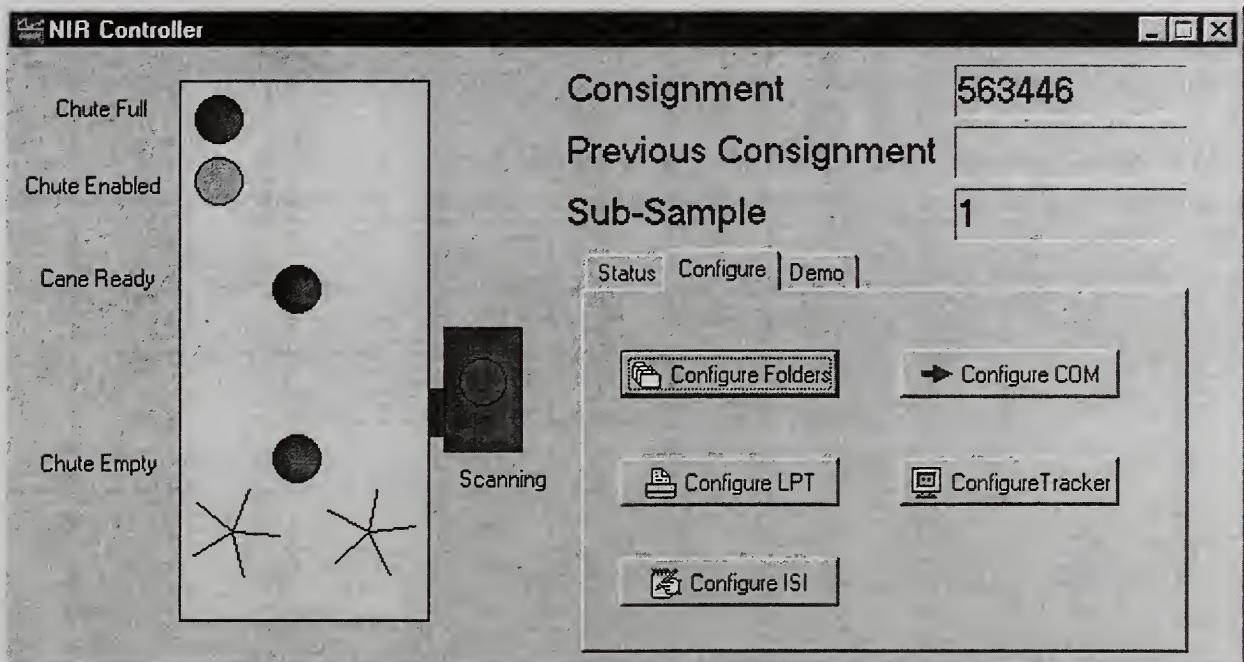


Figure 6. NIRS Control Program running together with ISIScan for autoscanning of shredded cane samples. Note mimic chute showing state of operation: *i.e.* state of sensors, motors, hatch and NIRS during operation. Red = device off or disabled, green = device on or enabled

Long term protection of NIRS calibrations

One of the major concerns of long-term NIRS is the transfer of calibrations, developed on a master or reference instrument to future NIRS instruments. Another concern is the possible inaccuracy of NIRS instruments after servicing or repair. In 2001, a NIRSystems 5000 had its wavelength encoder rebuilt. GH values of molasses samples were vastly different after the re-build ($GH > 50$) (Anon, 2002). Subsequent predictions were badly biased, see Table 6.

Fortunately molasses samples have been frozen and the NIRS was re-standardised. After optical matching, GH values dropped to normal ($1 < GH < 3$) with excellent predictions, virtually free from bias. Berding (2002) has recently given two examples of dispersive instruments producing highly biased predictions after service and repair. The occurrence of these breakdowns has rendered calibrations, developed over several years, virtually useless as reference materials for high moisture (high absorbing), unstable products are unavailable. The cost of these breakdowns, in terms of rebuilding the databases, is substantial. For this reason it is imperative that research into NIRS techniques be broadened to include

instruments that guarantee greater wavelength accuracy and precision. Process FT-NIRS spectrometers are rapidly establishing a reputation for excellent accuracy and precision, ruggedness and reliability. Since commissioning new instruments does not require optical matching, direct

transfer of calibrations is possible. It is imperative that dual monitoring of the NIRSystems 6500 (established, proven with potential wavelength repeatability and photometric problems) and FT-NIRS (state of the art, fast, reliable with high wavelength accuracy) be carried out on the same shredded cane samples. A Bruker Matrix F system has been identified as the most suitable FT-NIRS for online analysis of shredded cane. Funds have successfully been obtained for testing this system in the 2004/05 crushing season.

Table 6. SMRI calibration problem for molasses. Effect of replacing wavelength encoder.

Analyte	NIRS	Lab	Diff
Pol	32.0	29.5	2.5
Brix	76.4	83.4	-7.0
D.Solids	76.5	78.9	-2.4
Ash	8.4	15.1	-6.8

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THE APPLICATION OF NETWORK NIR CALIBRATION EQUATIONS AT THE MARYBOROUGH SUGAR FACTORY

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ABSTRACT

The work described in this paper includes the installation, commissioning and validation to successful performance of a FOSS Cane Analysis System (CAS) at the Maryborough Sugar Factory in 2003. It is noted that the system equations loaded contained no data from the Maryborough region or the NIR spectrophotometer used in the CAS. The results demonstrate that the transfer of FOSS based calibrations can be achieved with no loss in analytical performance.

The system was implemented at the Maryborough Sugar Factory to address cane quality issues, high processing costs, provide outputs for improved factory control and determine the effects to millers and growers of using the CAS for direct determination of CCS for payment purposes.

The system library required the addition of 350 valid spectra collected over roughly one week to adequately represent the new instrument and cane supply. Constituent biases were calculated and applied after the first week of operation and were the only adjustments made to the system. The precision of the CAS results was found to be within expected limits for all constituents except brix in cane and fibre; the reasons for the higher than expected error for these constituents are still under investigation.

The bias for all constituents was demonstrated to have a diurnal cycle and long-term drift. This effect was found to be due to variation in cane temperature and adjustment based on this parameter resolved the problem for all constituents.

The seasonal average for the current laboratory method for CCS was (15.12) using class fibre, and the CCS directly determined by NIR was (15.14), and as the reported errors were within expected system limits, there is no equity barrier to the implementation of direct NIR CCS in cane payment for the 2004 crushing season.

All stakeholders in the Maryborough cane-growing district agree in principle with this philosophy. The hurdle remaining is the difficulty in changing the cane payment formula to achieve a win-win scenario for all parties.

INTRODUCTION

Online near infrared (NIR) spectroscopy has been used to determine individual delivery fibre content for cane payment within the Australian sugar industry since 1998 and is described by Staunton *et al.* (1999). Since that time, the uses of the system have been expanded to include laboratory auditing, cane quality determination (Pope, 2004) and milling train process control (Jones, 2002).

The class fibre system, used by some Australian mills to assign a fibre value to each delivery for the calculation of Commercial Cane Sugar (CCS), is based on a three-day rolling average of laboratory determinations from composite snap samples collected for each class over a 24 hour period. Individual deliveries are assigned a fibre class according to the variety of the cane supplied. Varieties are usually grouped into classes on the basis of their contribution to the cane supply, e.g., more than 25% of supply into class Majors, more than 10% but less than 25% into class Minors, and all others into class Others. This method provides a very good estimate of the seasonal average fibre content but relates poorly to the fibre content of individual deliveries.

The NIR based analysis system was implemented at the Maryborough Sugar Factory to address the following issues:

- Cane quality. The current class fibre based payment method does not provide sufficient reward for growers providing high quality cane. The fibre value used is an average value based on the variety supplied, not the actual fibre of the delivery. The use of the class fibre payment system results in high quality producers subsidizing low quality producers through the average fibre assigned and may lead to incorrect conclusions regarding overall productivity.
- Processing costs. All deliveries to the Maryborough factory are via 24 tonne road transport. This results in a relatively high workload providing analysis for payment, approximately 230 per day. The use of the CAS system in direct CCS determination will reduce this analytical load by at least 90%, with 10% or less analysed for system validation. Reductions of extraneous matter in the cane supply will also contribute to a reduction in processing costs.
- Mill control improvement through reduced variation in the cane supply and utilization of CAS process outputs in automated control loops.

Recent papers by Berding (2002) and Schaffler *et al.* (2003) have stated that the transfer of calibration equations from one FOSS instrument to another or to one that has required repairs is not possible, resulting in the need to rebuild calibration sets at great cost. In our experience, this is not the case and the results presented in this paper serve to demonstrate that the transfer of FOSS based calibrations can be achieved with no loss in analytical performance.

SCOPE

The work described in this paper includes the installation, commissioning and validation to successful performance of a FOSS Cane Analysis System (CAS) at Maryborough Sugar Factory in 2003. It is noted that the system equations loaded contained no data from the Maryborough region or the NIR spectrophotometer used in the CAS. The routine practices completed during the commissioning and operation of the system can be broken down into three distinct phases:

1. Confirmation the system was collecting valid spectra and adjustment of the system spectral library to accommodate the new instrument and cane supply.
2. Determination of bias and standard error of constituent predictions and adjustment of system as necessary.
3. Determination of system key performance indicators (KPI) and monitoring of these parameters over the season. These include constituent error and bias and percentage of samples successfully analysed.

Another goal of this work was to determine the effects to millers and growers of using the CAS for direct determination of the CCS content of cane for payment.

The routine practical steps completing these phases are described below, together with relevant performance indicators.

EXPERIMENTAL

The FOSS Cane Analysis System incorporates a Direct Light 5000, post-dispersive, 1100 to 2500 nm NIR spectrophotometer within an environmental enclosure. The enclosure protects the spectrophotometer against vibration, temperature, humidity variation and incursions of dust and fibre. A Programmable Logic Controller (PLC) manages the quality of air, power and communications services to the system.

The system computer connects the PLC and NIR spectrophotometer and contains all system application software. It also acts as the factory interface for payment and process information and allows, with appropriate security, remote access for monitoring, control and support over a local or wide area network or the Internet.

The system scanner read-head is mounted on the chute of the 1st mill above and before the rollers and is connected to the system enclosure via a flexible anaconda cable. The system as installed at Maryborough is shown in Figure 1.

The CAS incorporated a subset of the Cane04 prepared cane network calibration set and the calibration statistics for the constituent equations used at Maryborough are detailed in Table 1.

The error control limit was calculated as 1.2 times the SECV generated during the calibration process and the bias control limit as 0.6 times the SECV.



Figure 1. Maryborough CAS system enclosure and read-head.

Table 1. Cane04 Calibration statistics

2003 Season Constituent	Calibration Statistics						Error Control Limit	Bias Control Limit
	SECV	R ²	Mean	Std. Dev.	Effective Range	N		
Brix % juice	0.28	0.99	21.27	3.11	11.9 - 30.6	4586	0.34	0.17
Pol % juice	0.32	0.99	19.03	3.06	9.8 - 28.2	3843	0.38	0.19
Fibre % cane	0.53	0.99	15.03	4.12	8.63 - 54.70	4054	0.64	0.32
CCS	0.33	0.98	14.23	2.35	7.1 - 21.3	3623	0.40	0.20
Pol % cane	0.28	0.99	15.15	2.83	0.6 - 21.6	3431	0.34	0.17
Brix % cane	0.25	0.99	17.69	2.18	11.2 - 24.2	3682	0.30	0.15
Ash % cane	0.37	0.73	1.63	0.71	0.00 - 5.67	3441	0.44	0.22
Dry Matter % cane	0.46	0.98	31.1	3.46	22.06 - 57.96	1696	0.55	0.28

Validation results for Pol in juice, Brix in juice and Fibre were determined using the methods contained in the Laboratory Manual for Australian Sugar Mills, Volume 2 (Anon., 2001). Dry matter and Ash were determined using methods developed by BSES for the calibration of the CAS systems.

Pol in Cane (PIC), Brix in Cane (BIC) and CCS are direct predictions by NIR and are not computations from the NIR analyses of the components. Laboratory results in these constituents are computed according to Australian cane payment protocols.

RESULTS AND DISCUSSION

1. System Installation

FOSS Pacific personnel installed the Maryborough CAS over a two-day period. The system read-head was positioned in the centre of the reverse face of the 1st mill chute approximately 0.5 metres above the rollers. Preparatory work by the factory included extension of an existing walkway for the system enclosure as well as the provision of single-phase power, factory air supply and data communication cables between the enclosure and the system computer. The system computer was situated in the milling train control room and communicated via RS232 protocols over a fibre optic link.

The system was switched on and sample scanning began on the afternoon of the 2nd day. Remote support of the system was possible via a www address on the Internet approximately one week after the system started scanning. Random problems were experienced with the operation of the fibre optic modems used in system communications approximately three weeks after operations began and were solved by the provision of new modems.

2. System Commissioning

System Spectral Library. The Cane04 spectral library was used to generate global (GH) and neighbourhood (nH) mahalanobis distance values for the spectra collected online in order to screen the data for corruption or excessive noise. The H values provide a measure of the compatibility between the substance being scanned and the spectra of samples incorporated in the system database. The structuring and maintenance of this library is critical to the system meeting its performance criteria for standard error of prediction and percentage of samples successfully scanned. Poor structuring and maintenance will result in valid spectra being rejected or corrupt spectra being accepted, the former adversely affecting the percentage of samples successfully scanned and the latter the standard error of prediction.

The Maryborough CAS used the network default library as the starting library for this project. This library contained spectra from several factories, instruments and seasons but no data from the Maryborough CAS. The GH and nH trends for the first week of operation are shown in Figure 1. The trends show the H values for the initial spectra collected at Maryborough were quite high with an average GH of 6.32 and nH of 4.38 as compared to the system acceptability limits of 3.0 for GH and 0.6 for nH. These values indicated the spectra were significantly different from those collected at other sites. It is normal routine to adjust the system library to account for this difference but it is crucial that only valid spectra are included and for this reason only an experienced person should attempt it. In this protocol, spectra only are added, and no analytical data are included so that the calibration equations are unchanged.

Before the system library could be adjusted, the collection of uncorrupted, noise-free spectra must be confirmed. This was achieved using the instrument diagnostic software supplied with the CAS and visual inspection of the spectra collected. These diagnostics use internal standards to determine the peak-to-peak noise, Root Mean Square (RMS) noise, bandwidth and the wavelength accuracy associated with the instrument. If the instrument passes the limits set for

these values the spectra collected can be deemed free of instrument noise and, if no obvious corruptions are noted in the visual inspection, then the spectra may be safely added to the system library. It is also useful at this point to check the constituent prediction trends. The results should show good repeatability for scans on the same consignment and step changes in the trend should correspond to a change of consignments.

After the system diagnostics confirmed noise free spectra were being collected, 50 spectra selected at random were added to the system library, the library was re-centered and the new library files were loaded onto the CAS. This process was repeated a further three times until the library was producing stable and lower H values. The application of each new library can be seen as a step change in the H value trends shown in Figure 2.

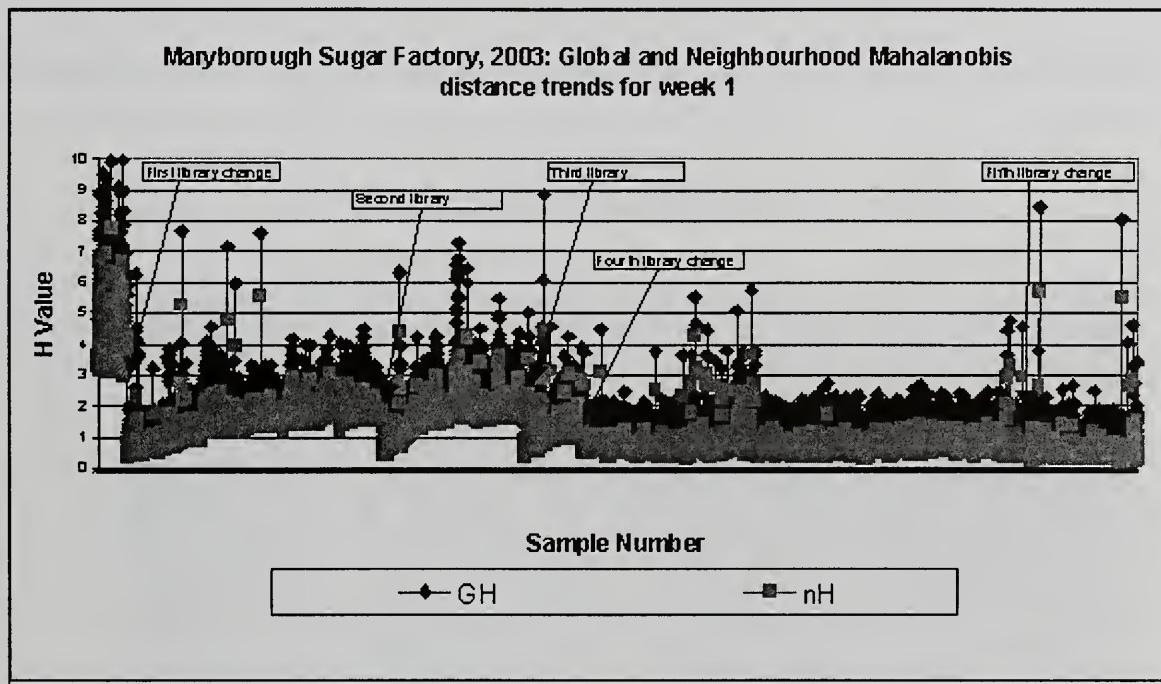


Figure 2. Mahalanobis distance trends for week 1.

Although there was no further drifting of the H values, many scans were still failing the nH limit, indicating there were insufficient close neighbours in the system library. Previous experience suggested that this was due to the temperature variation in the cane supply and a further 200 spectra were selected from this period in an attempt to represent the daily temperature variation. These spectra were added to the library and the new files were loaded onto the system. The effect of the new library can be seen in Figure 2 as the fifth step change in the H value trends.

After the fifth library was loaded, most scans were passing all system limits and the system operated with this library for the rest of the crushing season, about 80 days.

In summary, the system library required the addition of 350 valid spectra collected over roughly one week to adequately represent the new instrument and cane supply.

Constituent Equation Bias and Standard Errors

Once the system library was producing stable H values and effectively screening the data for corruptions, samples were collected, analysed and matched to the CAS results to determine the system bias and standard error of prediction (SEP) for each constituent. It was expected the system would have significant levels of bias as the initial H values indicated the system was significantly different from the calibration set but the standard error was expected to be within the control limits set for the system.

System bias was calculated as the average difference of the laboratory less the CAS result and the SEP was calculated as the standard deviation of the difference. The slope and correlation coefficient were determined using linear regression.

The validation statistics obtained for the sample sets used in determining system bias and SEP are shown in Table 2.

Table 2. Commissioning Validation Statistics

Constituent	SEP	Control Limit	Bias	Slope	R ²	N
Brix in Juice	0.32	0.34	2.00	0.93	0.90	628
Pol in Juice	0.30	0.38	0.30	0.95	0.92	628
Fibre	0.63	0.64	-0.47	0.76	0.63	29
Pol in Cane (Class Fibre)	0.32	0.34	0.88	0.98	0.87	628
Brix in Cane (Class Fibre)	0.43	0.30	2.93	0.95	0.77	628
CCS (Class Fibre)	0.37	0.40	-0.06	0.98	0.85	628
Dry Matter	0.72	0.55	-2.72	0.84	0.63	29
Ash in Cane	0.48	0.44	0.20	0.60	0.51	29

The validation statistics obtained confirmed the system to be operating within the control limits for all constituents except brix in cane, dry matter and ash. The statistics for dry matter and ash are based on small validation sets and were expected to improve with a larger sample set, the result for brix in cane was unexpected and appears to be the result of significant scatter in the predictions for this constituent. The cause of the scatter was thought to be the use of class rather than individual fibre in the calculation of this constituent. The statistics for pol in cane, brix in cane and CCS were expected to improve with the use of CAS fibre in the calculation of the laboratory values.

The bias adjustments for each constituent were applied by adjusting the results produced by each constituent equation by the value shown in Table 2. These were the only bias adjustments made to the system.

3. System operation

Determination and monitoring of CAS key performance indicators

After the system was aligned in the commissioning phase, it was important to validate the performance of both the system library and the constituent equations in on-line prediction. The system performance was monitored regularly and random check samples were used to highlight any problems that developed.

(i) Percentage of deliveries successfully scanned

The calculation of the percentage of deliveries successfully analysed on a daily basis, typically greater than 95% in the current cane payment method, was used to monitor the performance of the system library. The daily delivery analysis success trend for the entire period CAS was online at the Maryborough sugar factory is shown in Figure 3.

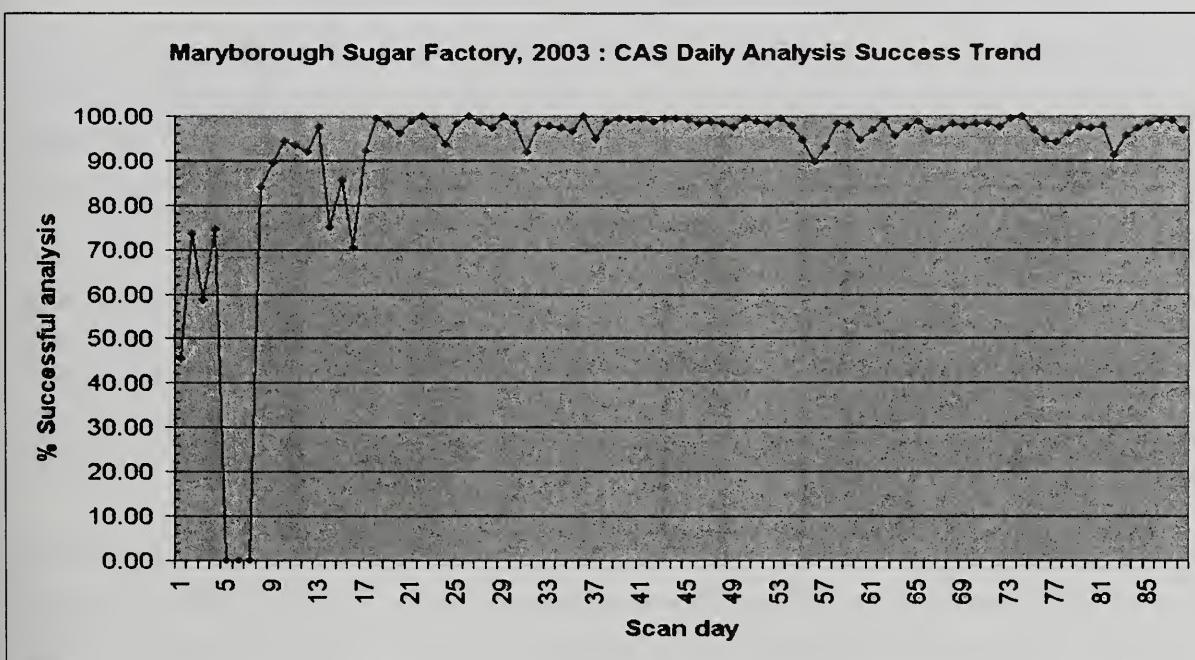


Figure 3. Delivery analysis success trend.

The trend shows the system operated with an analysis success over 90% for 75 days out of a possible 88 days, with an overall average of 94% for the trial. Those days where analysis success was below 90% can be attributed to the failure of the fibre optic modems used to communicate sample information to the system. The consistently high success rate over such a long period indicated the instrument and cane supply were well represented in the system library.

(ii) Constituent Equation Bias and Standard Error

Regular checking of the prediction results against the routine payment laboratory, typically three per day for fibre, and the plotting of these differences in a control chart format is the routine method used to monitor constituent equation performance in bias and standard error. The error

limit determined in the calibration process for each constituent was used to determine the warning and action limits on the charts.

Two types of control charts were used to monitor performance, namely, the average chart which was used to monitor system bias (accuracy) and the range chart which monitored system error (precision). The use of control charts allowed the user to quickly identify when the system was operating outside the expected levels of performance. This method of monitoring performance has distinct advantages over the more traditional method of validation, i.e., linear regression using an XY scatter plot. When using linear regression, extended periods of good performance can easily mask periods of poor performance, producing statistics that may be misleading. The use of control charts overcomes this problem as the system performance is presented as a trend line where periods of poor performance can be easily and promptly identified. The validation statistics obtained using linear regression for all deliveries analysed after the system was commissioned are shown in Table 3.

Table 3. Validation Statistics after bias adjustment

Constituent	SEP	Control Limit	Bias	Slope	R ²	N
Brix in Juice	0.36	0.34	0.03	1.00	0.91	12093
Pol in Juice	0.33	0.38	-0.04	0.95	0.92	12093
Pol in Cane (CAS Fibre)	0.29	0.34	0.15	0.97	0.91	12093
Pol in Cane (Class Fibre)	0.34	0.34	0.12	0.98	0.88	12093
Brix in Cane (CAS Fibre)	0.37	0.30	0.21	1.05	0.87	12093
Brix in Cane (Class Fibre)	0.46	0.30	0.19	1.02	0.80	12093
CCS (CAS Fibre)	0.38	0.40	0.09	0.90	0.87	12093
CCS (Class Fibre)	0.40	0.40	0.07	0.91	0.85	12093
Fibre	0.77	0.64	-0.05	0.76	0.59	93

The validation statistics obtained confirm the system to be operating within the error control limits for all constituents except brix in juice, brix in cane and fibre. The slope and correlation coefficients obtained from linear regression are acceptable for all constituents except fibre, which is expected to improve with the addition of more data.

The statistics also show the system experienced significant levels of bias for some constituents that cannot be explained by the use of class fibre in laboratory calculations, leading to the conclusion that the original bias adjustments were incorrect and the system required further adjustment. Inspection of the control charts, for CCS by CAS fibre as an example, reveals a greater depth of information than linear regression alone. The range control chart for CCS difference is shown in Figure 4. The chart shows the CCS prediction by CAS had very good precision over the entire project with only 46 (1.14%) breaches of the warning limit and 9 (0.22%) breaches of the action limit

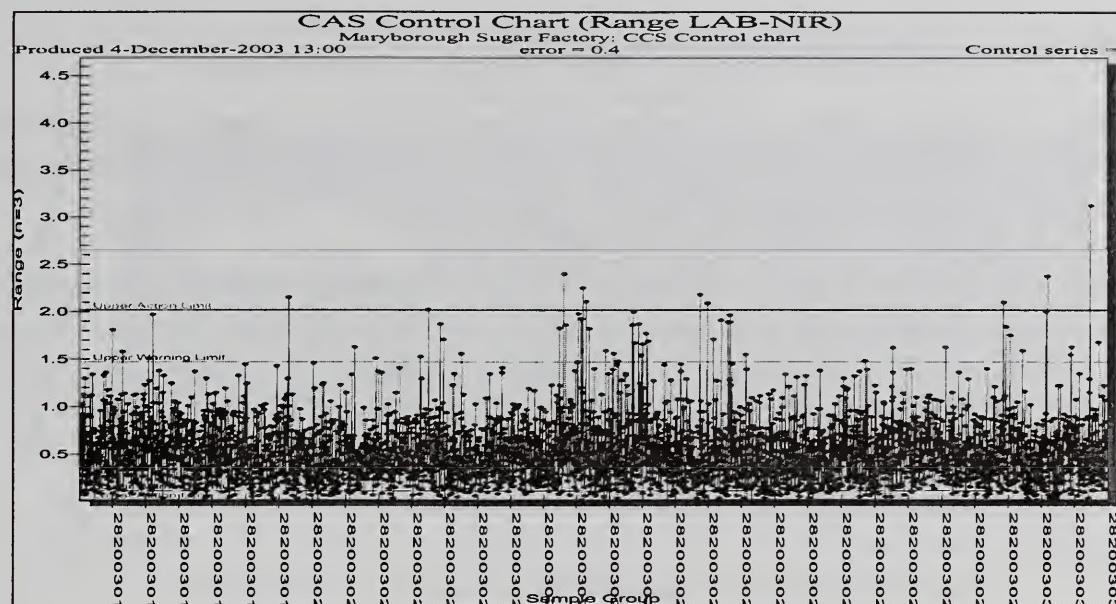


Figure 4. CCS range of difference control chart.

The average control chart for CCS difference is shown in Figure 5. This chart deals with the average difference between a laboratory result and the CAS or bias. It can be seen from the trend that the CCS bias was incurring a diurnal cycle and that it also drifted over time, through the acceptable range of differences, to breach both the upper and lower action limits on several occasions.

This effect has been observed at other sites and the method of minimising system bias has been to regularly adjust the bias correction to maintain the trend within the warning limits. Research completed towards the end of the 2002 season pointed to the temperature of the cane (or ambient) as the main driver of this bias drift and as a result no further adjustments were made to the system during its operation to test this theory.

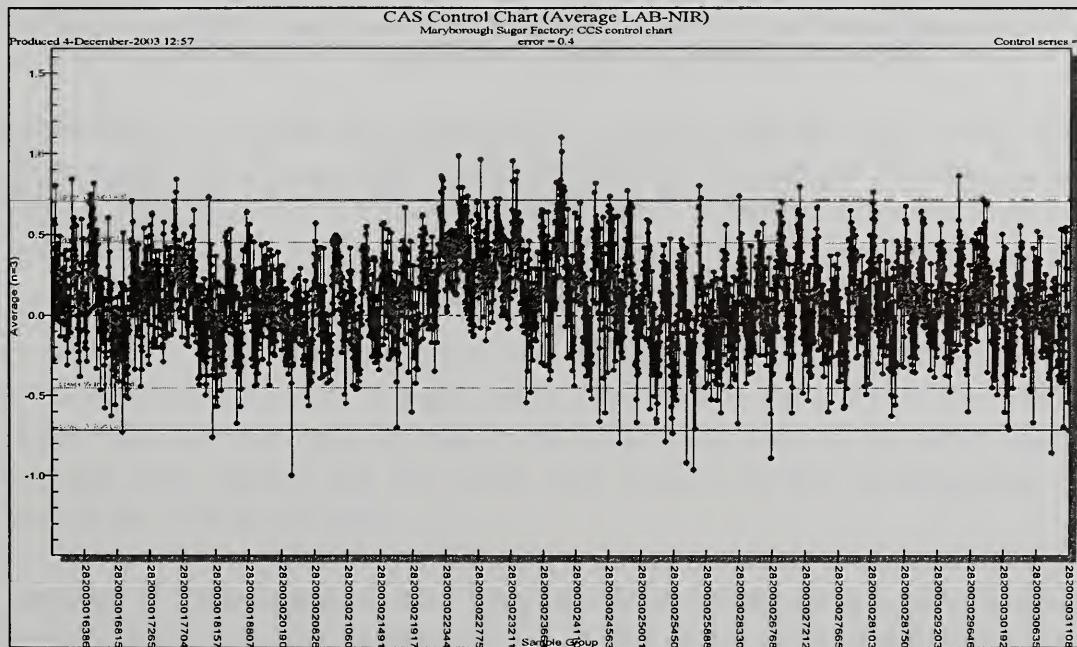


Figure 5. CCS average difference control chart.

Investigation into the effect of Sample Temperature

A three sample rolling average juice temperature trend for the season was generated and is shown in Figure 6, where the middle line is the season average temperature and the upper and lower lines are two standard deviations from the average.

The trend for temperature exhibits the same diurnal cycle and drift as the average difference control chart for CCS, indicating it as a significant, if not the main driver for bias variation. The two trends were so similar that an adjustment to CAS CCS based on the cane temperature would likely address most of the bias issues in determining CCS directly by CAS. A further benefit that accrues is a reduction in the SEP that will have been affected to some degree by the variable bias.

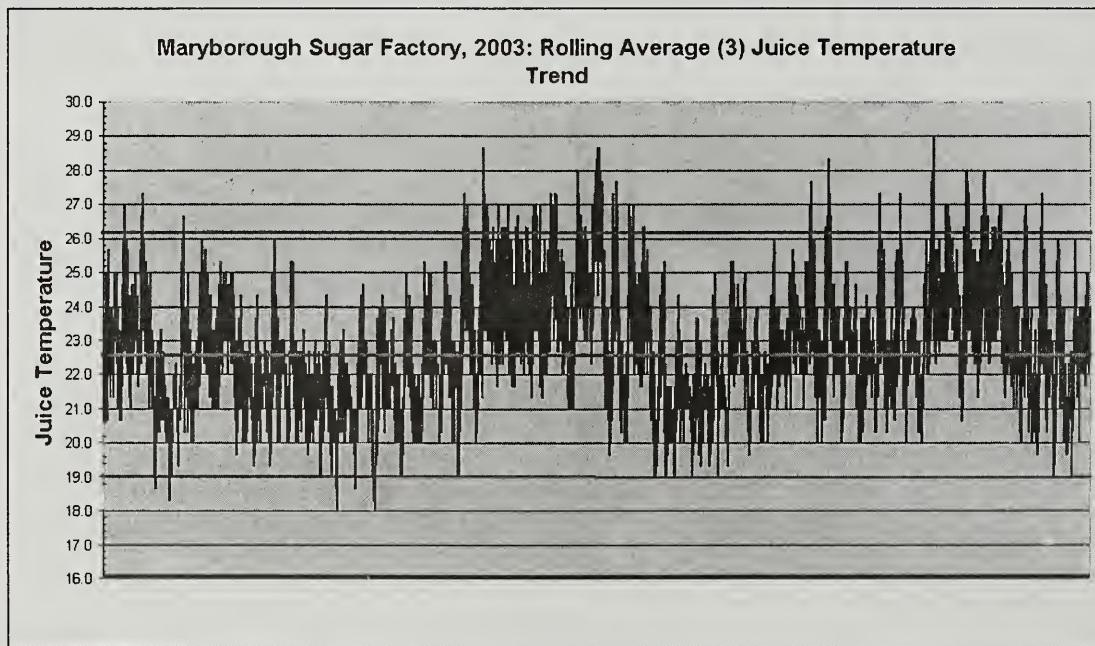


Figure 6. Rolling average juice temperature trend.

The calculation of a CCS adjustment due to temperature was based on the regression equation obtained from a plot of average CCS difference (Lab – CAS) against juice temperature rounded to the nearest unit, shown in Figure 7. Using this relationship a fine-tuning CCS adjustment was calculated from the measured juice temperature, applied to the CAS result and the validation statistics were regenerated for the adjusted result.

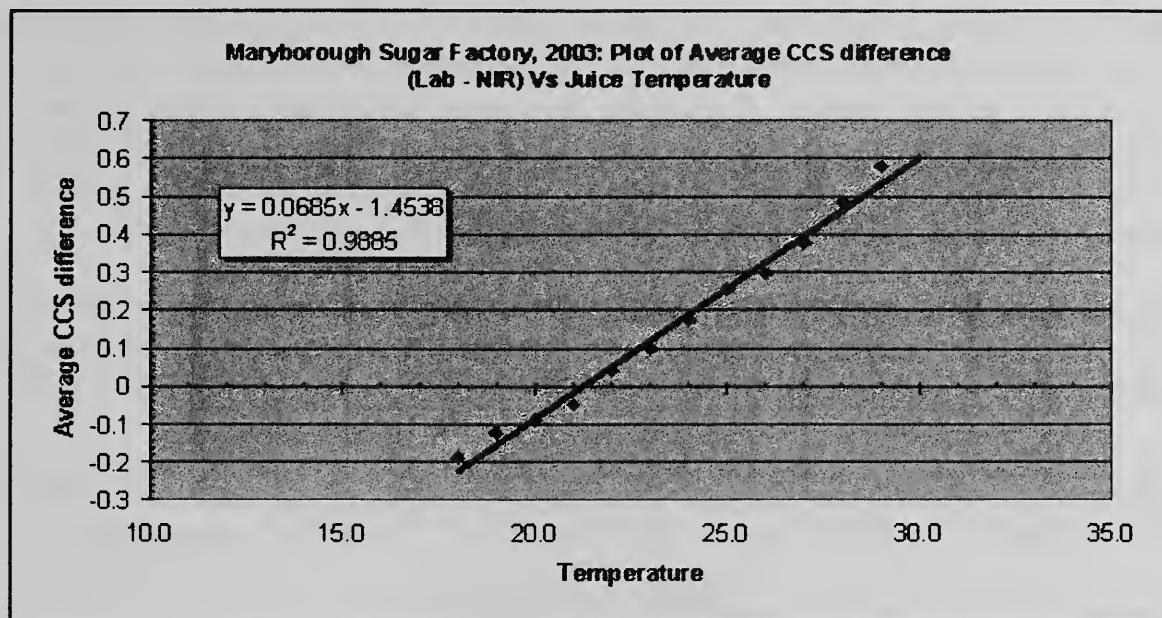


Figure 7. Average CAS CCS difference against juice temperature.

The same methodology was applied to the other constituents predicted by the system and the corrected validation statistics are shown in Table 4.

Table 4. Validation Statistics corrected for temperature

Constituent	SEP	Control Limit	Bias	Slope	R ²	N
Brix in juice	0.34	0.34	0.00	1.00	0.91	12093
Pol in Juice	0.32	0.38	0.00	0.96	0.93	12093
Pol in Cane (CAS Fibre)	0.28	0.34	-0.01	0.97	0.92	12093
Brix in Cane (CAS Fibre)	0.37	0.30	-0.01	1.05	0.88	12093
CCS (CAS Fibre)	0.36	0.40	-0.02	0.92	0.88	12093
Fibre	0.74	0.64	-0.01	0.78	0.61	93

The statistics in Table 4 show the adjustment of the CAS results based on the methodology described above addresses the bias issues found for CAS predictions. As expected, there is a minor reduction in the SEP.

The adjusted results show the system to have met the control limits for all constituents except brix in cane and fibre. The situation in the case of fibre appears to be one of skew, i.e., over predicting at low values and under at high values, and may be the result of the small validation set. The higher than expected brix in cane is out of keeping with all the other observations on the Maryborough cane supply and the issue will require further investigation both at the laboratory and in the NIR predictions.

This work reinforces the conclusion that, after the initial set up of the system, bias variations for all parameters predicted are attributable to variations in the cane temperature.

The ability to calculate system bias adjustments based on the temperature of each delivery reduces the average difference between the two payment methods to near zero, minimising any change in miller – grower equity. Comparing the current laboratory analysis method (15.12) using class fibre, with the average CCS directly determined by NIR (15.14) and given that the reported errors were within expected system limits, there is no equity barrier to the implementation of direct NIR CCS in cane payment for the 2004-crushing season.

All stakeholders in the Maryborough cane-growing district agree in principle with this philosophy. The hurdle remaining is the technical considerations in changing the cane payment formula to achieve a win-win scenario for all parties.

The addition of data from the Maryborough system into the network calibrations for use in season 2004 is expected to greatly reduce the bias corrections required.

CONCLUSIONS

The Cane Analysis System (CAS) was successfully installed and commissioned at the Maryborough Sugar Factory. The system successfully analysed 94% of the cane deliveries while in operation. The precision of the CAS results were found to be within expected limits for all constituents except brix in cane and fibre, the reasons for the higher than expected error for these constituents are still under investigation.

The bias for all constituents was demonstrated to have a diurnal cycle and long-term drift. This effect was found to be due to variation in cane temperature and adjustment based on this parameter resolved the problem for all constituents.

The results obtained demonstrate that the transfer of FOSS based calibrations to new or changed instruments can be achieved with no loss in analytical performance.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the contribution of Keith Cooper from FOSS Pacific for his hard work during the installation of the CAS and thank the Board of the Maryborough Sugar Factory for granting permission to publish these results.

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MEASURING SUGAR LOSSES IN BRAZILIAN MILLS

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ABSTRACT

Brazilian mills produce 14.4 billion liters of ethanol and 24.2 million tons of sugar per year. Many factors affect industrial efficiency, but the losses of sugar are very significant. Without measuring the losses, it is impossible to control and to manage the factory with high efficiency. The main losses measured in Brazilian mills are the amounts of sugar in sugarcane wash-outs, filter cake, barometric column and residual waters. During the last harvest season, the losses varied from 0.06 to 1.05% of sugar in sugarcane wash-outs, 0.19 to 2.60% in filter cake, 0.01 to 1.49% in barometric columns, 0.025 to 1.78% in residual waters and 2.5 to 8.0% in bagasse (extraction). Data is presented on sugar destruction at evaporators (pre-evaporation to final syrup), where there are losses up to 3.5% of the sugar in cane.

INTRODUCTION

Brazil is the largest worldwide producer of sugar and ethanol from sugarcane. During the 2003 harvest season, 324 industries crushed 350 million tons of sugarcane, produced 24.2 million tons of sugar and 14.4 billion liters of ethanol. The laboratory control performed in the mills is fundamental for the factory to improve the performance and the quality of their products. This control ranges from sugar determination (POL), reducing sugars (glucose and fructose) and total reducing sugars (sucrose, glucose and fructose). Because of the fact that glucose and fructose are used by yeast to produce ethanol, in Brazil it is used to determine the sugar balance in the factory and to measure TRS (total reducing sugars). Fermentec has developed and adopted analytical methodologies to measure total reducing sugars in low amounts in bagasse, filter cake and waters used in the industry. When losses occur by sucrose inversion, the efficiency of the

sugar factory will be reduced, but the efficiency of the distillery should be the same or unaltered because yeast cells are able to use sucrose, glucose and fructose to ferment.

Many factors may affect the industrial efficiency, but sometimes the losses of sugar are very significant. Without measuring the losses, it is impossible to control and to manage the factory with high efficiency. This article describes how Brazilian mills have analyzed the sugar losses, where are the main sources of sugar losses, and which had been the variations observed in 56 mills during the 2003 harvest season. Knowing the sugar loss, it is possible to identify the cause (reason) and to curb the loss.

SAMPLING AND METHODOLOGIES FOR SUGAR DETERMINATION

The first aspect to consider in control of sugar losses is how to sample and how to measure low sugar amounts with high precision and accuracy, at low cost. During the last 10 years Fermentec has developed new ways of sampling in partnership with Brazilian mills. These procedures are based on continuous sampling of juice, cane bagasse and waters during the process of extraction, juice evaporation, as well as in other steps. Moreover, we have observed the importance of sample conservation during the sample collection because of microbiological sugar decomposition. By this procedure, we have obtained adequate samples to determine sugar losses.

The second aspect to be mentioned is the choice of methodology to be applied to sugar determination in dilute samples. From samples of wash water and barometric columns, we have used an adaptation of the Somogyi-Nelson Method for reducing sugar determinations as low as 6 ppm (Amorim et al., 1979). For juices, syrups and molasses we have developed the equipment called REDUTEC that determines total reducing sugars (Zago et al., 1992). This equipment is based on an oxy-reduction electrode that increases the precision of the titration, reducing the analyst error. Ideal would be an HPLC or a gas chromatograph, but this apparatus is very expensive, and for juice it is not essential. For molasses it is indispensable, because neither POL nor TRS accurately measures the sugar content, and it is impossible to make an accurate sugar balance in the factory. Besides, the use of NIRS (Near Infrared System) brought new possibilities to sugar determination, cane payment and many other routine analyses. The NIRS was introduced in the Brazilian mills in 1993 and nowadays nine customers have been using it. One mill uses it on shredded cane (reflectance) for cane payment and the others use it for POL in juice (absorbance), also for cane payment. Most of them use the NIRS also for all the analyses in the distillery, such as ethanol, glycerol, percent yeast, and many others with the exception of pH and acidity.

SUGAR LOSSES IN BRAZILIAN MILLS

During the last ten years, due to research in many mills, the factories changed their method of cane storage. Before, the burned stalks of sugarcane were stored in the patio in large piles until crushing. In that time, the sugar losses were very high because of excessive transport and crushing of the stalks by trucks and tractors, as well as by microbial deterioration of the burned sugarcane. Experiments developed in Da Pedra Mill showed these losses could reach up to 15% of the total reducing sugars of the cane. However, it depended on the crushing method and

washing (Figure 1). Besides, another very important factor to be considered is the percent of fiber in the cane.

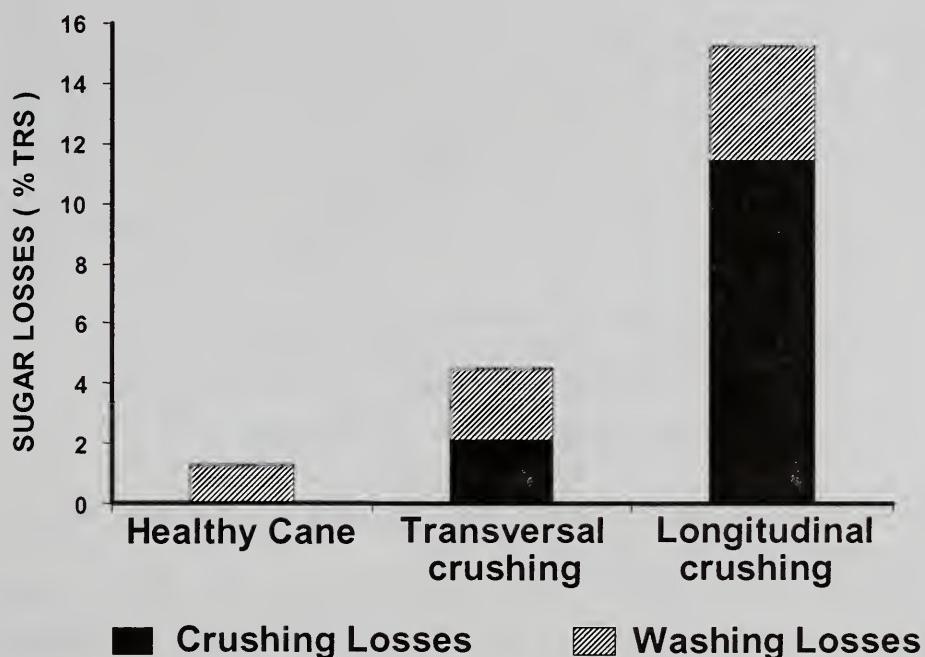


Figure 1. Determination of sugar losses by cane crushing and washing in Da Pedra Mill. Transversal and longitudinal represent the method of crushing the stalks by tractors.

Sugarcane varieties have different amounts of fiber conferring better or poorer resistance to breaking. High fiber in cane reduces the sugar extraction efficiency but reduces the sugar losses in cane wash water. On the other hand, low fiber in cane increases sugar extraction but also increases sugar losses in cane wash water (Figure 2). It is important to avoid excessive crushing and storage of the cane stalks in cane yards and large piles if the industries are milling a sugarcane variety low in fiber. The deterioration and losses are higher.

Nowadays, many industries have reduced or eliminated their patio (cane yard) and piles of sugarcane storage. The main results achieved were the reduction of the sugar losses, better raw cane quality and less impact on industrial yield. In the 2003 harvest season, the average of sugar losses among the mills accompanied by Fermentec was 0.99 % TRS cane, but the variation was between 0.06% to 3.93 % TRS cane. A case study realized at Alcidia mill, in 1990, demonstrated that sugar losses could reach 265 Kg/h in the heavy conveyor. The losses were measured at different points of the conveyor during crushing. The main sugar losses occurred in the region of the knives (until 100 Kg TRS/h). This loss accounted for 0.57% of the total sugar entering the mill.

Another important source of sugar losses is the filter cake. Factors that affect the production of the mud and cake also increase the sugar losses. An example is the amount of soil in cane. It is demonstrated that when soil in cane increased, the production of filter cake increased (Figure 3), increasing the loss (Figure 4). In 2003, the average sugar loss in filter cake was 0.62 % TRS cane, while the variations were between 0.12 to 3.57% TRS cane.

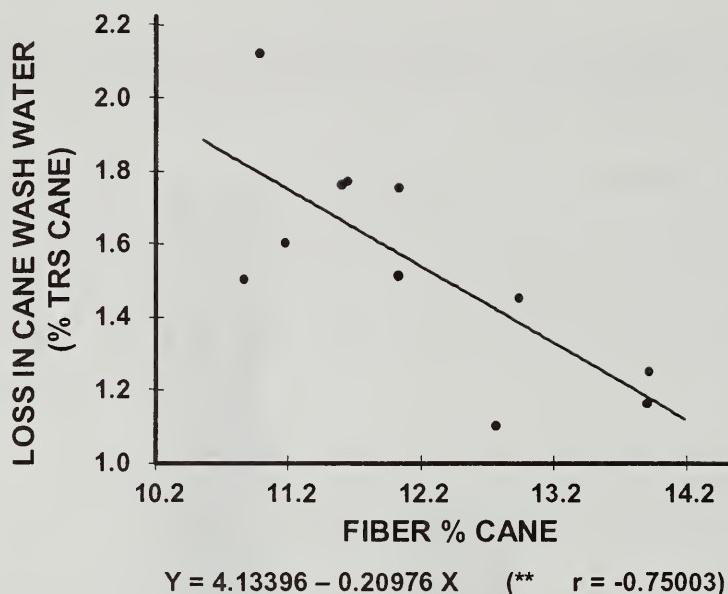


Figure 2. Effects of % fiber in cane on sugar losses in cane wash water. The data represent weekly averages from Melhoramentos mill (1999-2000).

Sometimes it is difficult to convince the plant staff about the dimension of the losses when they don't measure and don't believe that the loss can be significant. This has been observed for sugar losses in floor wash waters. Recently, the number of mills measuring the sugar losses in floor wash water has increased. In the last harvest season, 37 mills have been measuring it. The average loss in floor wash water was 0.325 % TRS cane, while the variations were between 0.018 to 1.62 % TRS cane. Another important source of losses is in the barometric column (last effects, vacuum pan and filters). In 2003, the average of these sugar losses was 0.278% TRS cane. The variation observed was between 0.014 to 2.230% TRS cane. In many mills, the bacterial contamination has a direct effect to losses in barometric column. The dextran formed by *Leuconostoc mesenteroides* increases the losses in the barometric column (Figure 5).

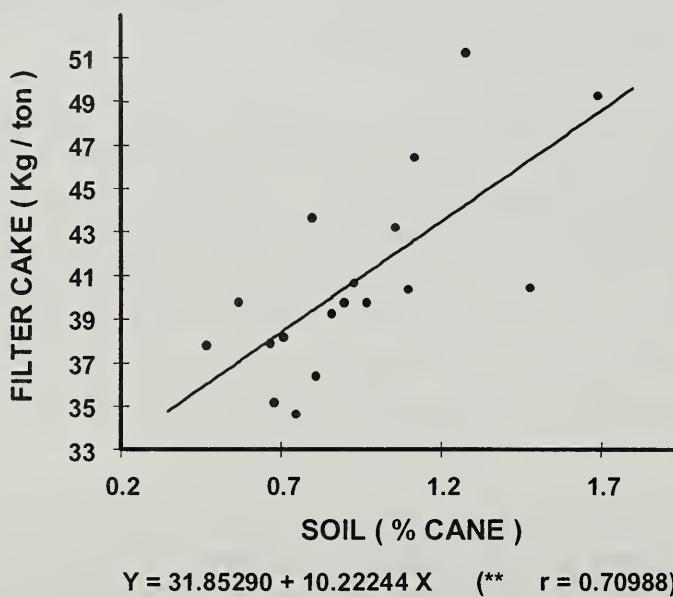


Figure 3. Effect of soil (% cane) on quantity of filter cake produced. The data represent weekly averages from Colombo mill (2000-2001).

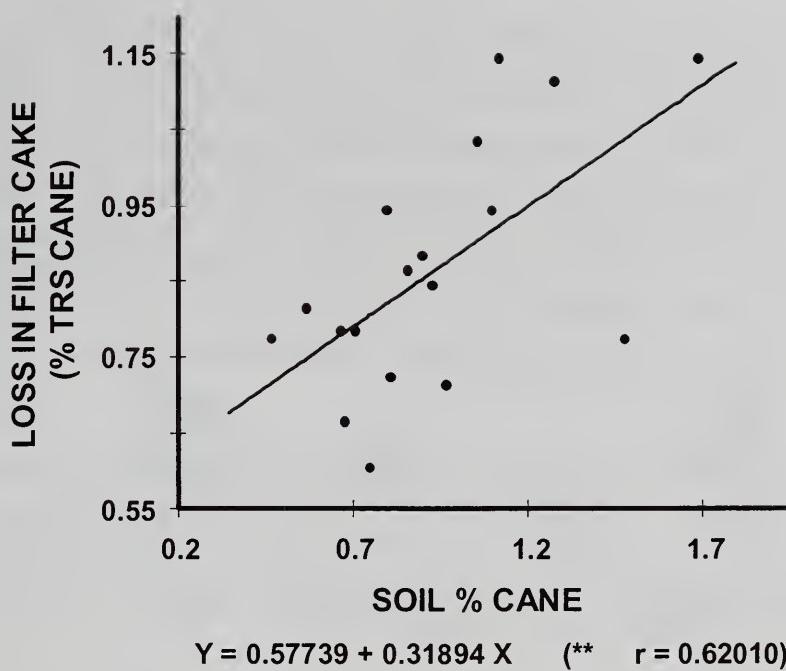


Figure 4. Effect of soil (% cane) on sugar losses in filter cake. The data represent weekly averages from Colombo mill (2000-2001).

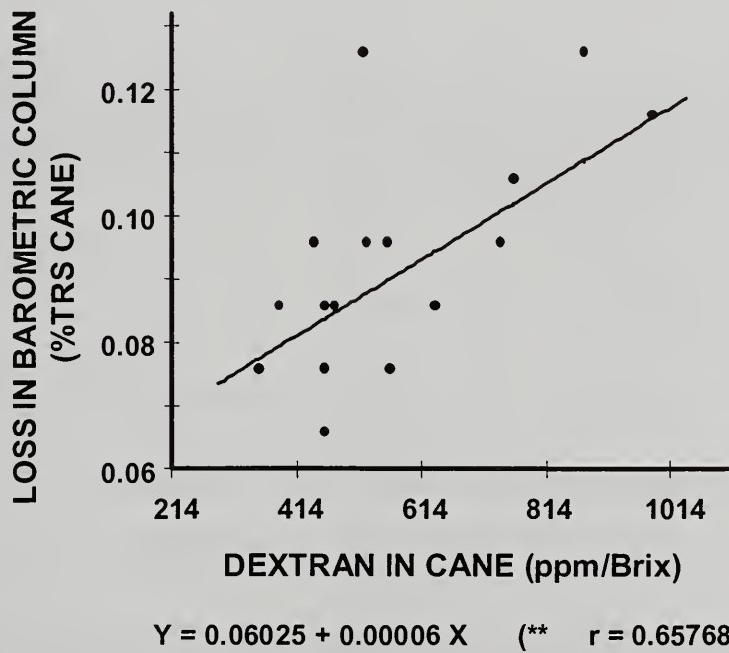


Figure 5. Effect of dextran in cane (ppm/Brix) on sugar losses in barometric column. The data represent weekly averages from Batatais mill (2000-2001).

Furthermore, sugar losses can be increased by bacterial rods, such as *Lactobacillus* and *Bacillus* species, that contaminate sugarcane juice with very high frequency (Figure 6). Bacterial contamination and dextran increase the viscosity of the massecuites, decrease the evaporation rate and if one forces the flow, the probability of a spill increases and this also increases the loss.

Another aspect that needs be pointed out is sugar destruction. In this case, there is a difference between sucrose inversion and sugar destruction. Inversion means a sucrose loss, but destruction is the complete loss of glucose and fructose by reactions that prevent their use to produce ethanol by the yeasts. In the last years, efforts have been made to identify sources and indicators for cane juice deterioration and sugar destruction under processing conditions (Eggleston, 2002; Andrews et al., 2002). During the 2003 harvest season, we started a study with 7 Brazilian mills to identify sugar losses in the evaporators (pre-evaporators, first effects and syrups). The results obtained demonstrated that sugar destruction occurred, not only by sucrose inversion, but also by reactions with glucose and fructose (Figure 7). These results demonstrated the importance of measuring sugar losses and destruction in the industries.

Factors such as raw sugarcane quality and the sugar losses affected the industrial yield (Amorim et al., 2000). Fermentec has followed 56 Brazilian mills in the 2003 harvest season looking to identify the main factors that affect the industrial yield. The mills classified in the best group (A) achieved the best results on the sugarcane quality and lower sugar losses as compared with mills classified in groups B and C, as demonstrated in Tables 1 and 2.

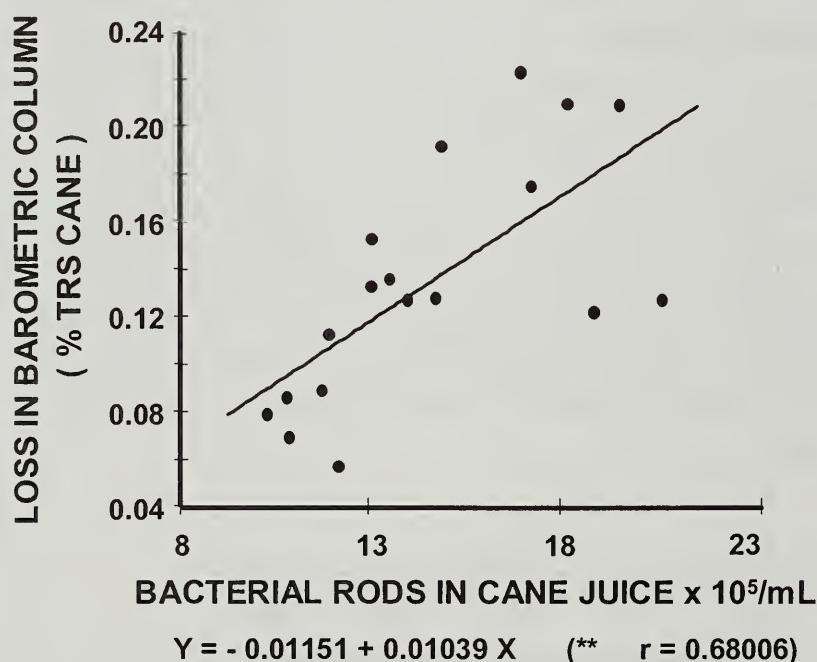


Figure 6. Effect of bacterial rods in cane juice on sugar losses in barometric column. The data represent weekly averages from Alta Mogiana mill (1998-1999).

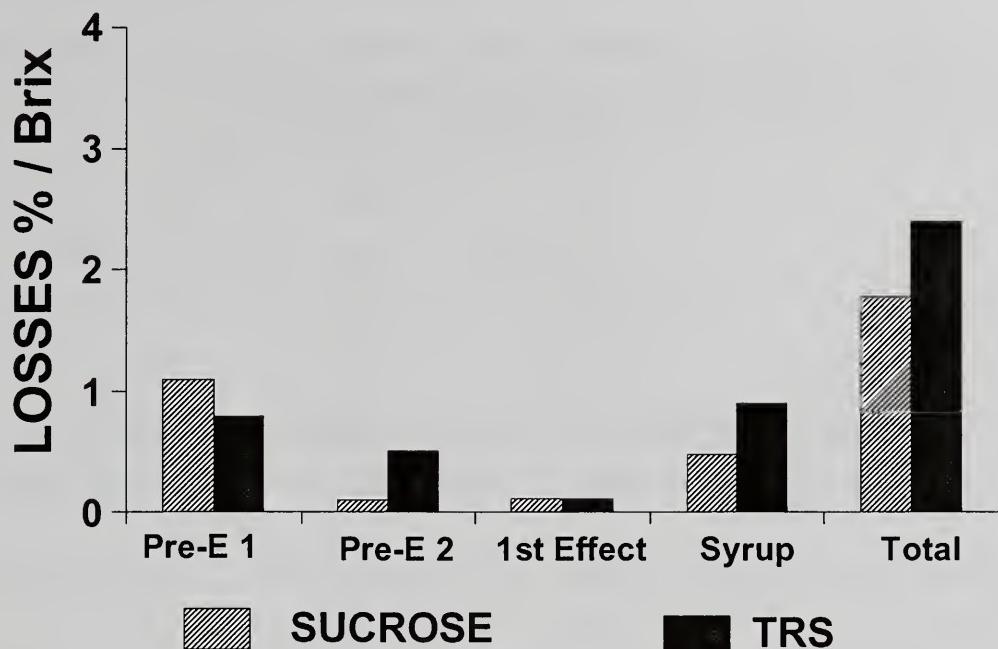


Figure 7. Sugar destruction measured as sucrose and total reducing sugars (TRS) in pre-evaporators, first effect and syrup. Data from Santa Elisa mill on July 4th, 2003 (Amorim et al., submitted to International Sugar Journal).

Table 1. Impact of the raw sugar cane quality on industrial yield of 56 Brazilian mills in 2003. The mills were classified into 3 groups (A, B and C) according to their industrial yields.

Raw Sugar Cane Quality	INDUSTRIAL YIELD		
	A (92-94%)	B (89 – 91%)	C (< 88%)
Dextran in cane (ppm / Brix)	476	660	722
Hours from burmnning to crush	38.2	42.8	50.3
% Alcool / Brix in the cane juice	0.26	0.28	0.33
Rods in the cane juice ($\times 10^5$ /mL)	3.5	5.0	6.1
Minerals (soil) Kg / ton	7.0	8.3	9.3

Source: Fermentec 2003

Table 2. Impact of sugar losses on industrial yield of 56 Brazilian mills in 2003. Mills were classified into 3 groups (A, B and C) according to their industrial yields.

Sugar Losses	INDUSTRIAL YIELD		
	A (92-94%)	B (89 – 91%)	C (< 88%)
Extraction (% TRS cane)	96.4	96.1	95.4
Cane wash water (% TRS cane)	0.50	0.94	1.34
Floor wash water (% TRS cane)	0.19	0.25	0.52
Barometric column (% TRS cane)	0.20	0.34	0.48
Filter cake (% TRS cane)	0.47	0.51	0.88
Fermentation Yield (%)	90.6	89.6	89.1

Source: Fermentec 2003

FINAL THOUGHTS

Concerning sugar losses we can affirm that to manage a factory well, it is necessary to have trustworthy information. To have trustworthy information, it is necessary to have an adequate system of sampling, analytical methods, equipment and trained staff. As Peter Druker said “*What you do not measure, you cannot manage.*”

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NEW INSIGHTS ON SUCROSE LOSSES ACROSS FACTORY EVAPORATORS AND JUICE AND SYRUP CLARIFIERS

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ABSTRACT

A major aim of an Agricultural Research Service project of the United States Department of Agriculture has been to reduce industrial sucrose losses. This paper discusses new insights gained from this project on sucrose losses in sugarcane juices and syrups at high temperatures (55-115°C) during different evaporation and clarification processes. Gas chromatography was used to determine glucose and sucrose concentrations on a °Brix or % chloride basis. Comprehensive studies were conducted at Louisiana factories to determine the effects of time between cleaning of Robert's-type calandria and plate evaporators on sucrose losses and overall evaporator performance.

In general, for both factories most sucrose inversion losses occurred in the pre-evaporators and were more a function of temperature, heating surface, °Brix and pH than retention time (R_t). Sucrose inversion only occurred in later evaporator bodies when scale had built up after a clean-out and, generally, became worse until the next clean. Less inversion occurred across the low R_t plate evaporator, although the plate was increasingly susceptible to scaling, but to a lesser extent than the Robert's type evaporators. Increasing the factory target pH of the clarified juice (CJ) or final evaporator syrup (FES) systematically reduced losses of sucrose and a target FES pH of ~6.3-6.5 (equivalent to a target CJ pH of ~ 7.0-7.3) is recommended; however, scaling effects can override pH effects. Seasonal effects on sucrose losses were also marked, and a higher target CJ or FES pH is recommended in early season or when processing immature cane. There was a consistent increase in pH in the last evaporator bodies because of the evaporation of volatile acids into the condensates.

Economic implications of sucrose losses are described. Sucrose losses across a Florida, U.S. factory's juice and syrup clarification processes were also investigated. In the juice clarifier, chloride trended with °Brix and, therefore, either could be used as a sucrose loss reference. In comparison,

in the syrup clarifier °Brix was more preferentially destroyed than chloride, and underestimated sucrose losses. High operational pHs for juice hot (~200°F or 93.3°C) lime clarification (pH range = 8.4-9.6 measured at room temp.) minimized sucrose losses to ~0.0-0.6% even with an estimated R_t of 3h, but excessive lime addition can accelerate color formation, scaling, and associated sucrose losses in evaporators. Higher acidic conditions (pH range = 6.3-7.4) across syrup clarification (phosphatation) caused much higher sucrose losses of ~0.6-1.3% even at a lower R_t and temperature of ~1h and 165°F (74°C), respectively.

INTRODUCTION

Millions of U.S. dollars are lost each year in the U.S. sugar industry because of the chemical loss of sucrose during harvesting, transportation, and processing. Moreover, there are associated detrimental effects from sugar degradation products formed. Degradation products can increase the loss of sucrose to molasses, decrease process efficiency, increase unwanted color, and decrease end product quality.

The high temperature conditions of clarification and evaporation processes are conducive for some of the highest sucrose losses in raw sugar manufacture. Furthermore, evaporators are often susceptible to scaling problems and the impact of scaling on sucrose losses has never been determined. Factory staff must consider all costs to make reasonable economic decisions on when to clean the evaporators' heat exchangers, and this also should include the impact of scaling on sucrose losses. Optimization of the time between cleaning should also include when the mean hourly cost of sucrose losses achieves its minimum value (Putman, 2001). Currently in U.S. sugarcane factories, the evaporators of choice are Robert's-s-type calandria evaporators, which are considered to be simple, robust and easy to operate. However, the tubular heat exchangers are susceptible to scale and require cleanings, often referred to as "wash-outs" or "boil-outs". Average reports of the length of time between evaporator cleanings for Louisiana factories are approximately 8-10 days, but 2.5 days have been reported in Florida factories.

Scale formation occurs because of the concentration of non-sugars across the evaporator station, especially the later evaporators where some of the inorganic non-sugars become supersaturated, precipitate out, and deposit on the heating surfaces (Honig, 1963). As inorganic non-sugars vary from region to region, the nature of scalants vary, too. Recently, Godshall and Wartelle (2002) reported that scale in Louisiana was mostly calcium and silicate. Calcium was highest in first and second effect evaporators, phosphorus peaked in the second and third effects and silicate generally increased across the evaporator station (Godshall and Wartelle, 2002). Scaling acts to reduce the overall heat transfer coefficient (U) in multiple-effect calandria evaporator stations. A scale thickness of only 0.2 mm can reduce U to almost half (Helmut and Joachim, 2003), and silicate scale causes the highest reduction in heat transfer and is the most difficult to remove. Furthermore, heat transfer coefficients are dependent on a number of other factors, including solution velocity, viscosity, and temperature (Chen, 1993).

In clarifiers and evaporators, sucrose is usually lost through thermally-catalyzed acid degradation/inversion reactions or physically by vapor entrainment, although the latter is much less than the former. Acid degradation or inversion of sucrose is a misnomer because it can occur up to ~ pH 8.3 (Parker, 1970). It can also be catalyzed by glucose, fructose (Richards, 1988) and/or salts (Richards, 1988; Eggleston et al, 1996), and this is becoming a bigger problem with the worldwide change to green cane from burnt cane, which has greater amounts of such associated impurities. In the few sucrose loss studies that have been undertaken across evaporators in South Africa (Schaffler et al, 1985; Purchase et al, 1987), the U.S.A. (Edye and Clarke, 1995), and Mauritius (Wong Sak Hoi and Tse Chi Shum, 1996), it was generally observed that most inversion occurred in the pre-evaporators. This is not surprising as the highest temperatures and lowest °Brix values occur there, which are both conducive to higher inversion rates. Pre-evaporators are usually larger to produce vapor for juice heaters and vacuum pans. However, Purchase et al (1987) found a positive correlation between the relative size of the pre-evaporators and losses.

The measurement of sucrose losses across unit processes in the sugar industry is notoriously difficult, and this has also meant that very limited diagnosis of process problems contributing to losses has occurred. Sucrose levels are traditionally measured at the factory using pol purity measurement, but pol cannot be used to measure small sucrose losses as degradation products formed with a high positive pol suppress the overall pol changes due to losses (Eggleston et al, 1996). Even using the more accurate techniques of high performance liquid chromatography and gas chromatography is difficult (Schaffler et al, 1985; Purchase et al, 1987; Edye and Clarke, 1995; Wong Sak Hoi and Tse Chi Shum, 1996), because actual sucrose loss may be smaller than the experimental error of the technique being used. Furthermore, the relatively large amounts of sucrose present, compared to the smaller amounts of glucose and fructose, also contributes to the greater difficulty in measuring actual sucrose decreases. As a consequence, sucrose losses are more easily measured indirectly from Δ%glucose/%sucrose ratios (Schaffler et al, 1985) according to the following formula:

$$\text{% Sucrose lost} = \frac{\left(\frac{(\text{% Glu})_{\text{out}}}{^{\circ}\text{Brix}} - \frac{(\text{% Glu})_{\text{in}}}{^{\circ}\text{Brix}} \right) \times \text{MW}_{\text{Suc}} \times 100}{\frac{(\text{% Suc})_{\text{in}}}{^{\circ}\text{Brix}} \times \text{MW}_{\text{Glu}}}$$

where MW = molecular weight, Suc = sucrose, and Glu = glucose

Glucose is used instead of fructose because it is more acid and heat stable. % Chloride has been used as a reference marker instead of °Brix because some dissolved solids may be degraded into volatile compounds (Schaffler et al, 1985; Wong Sak Hoi and Tse Chi Shum, 1996), but Wong Sak Hoi and Tse Chi Shum (1996) observed that there was a constant °Brix/% Chloride ratio in evaporator juices and syrups which implied that °Brix is not preferentially destroyed to % chloride. Therefore, either °Brix or % chloride can be used as a reference marker in evaporator studies.

$$\% \text{ Sucrose lost} = \frac{\left(\frac{(\% \text{ Glu})_{\text{out}} - (\% \text{ Glu})_{\text{in}}}{\% \text{ Cl}} \right) \times \text{MW}_{\text{Suc}} \times 100}{\frac{(\% \text{ Suc})_{\text{in}} \times \text{MW}_{\text{Glu}}}{\% \text{ Cl}}}$$

Measurements of sucrose losses based on Δ%glucose/%sucrose ratios, however, are still underestimates. This is because they are based on the assumption that no glucose is degraded, although Schaffler et al (1987) reported that even under adverse acid conditions, compensation for glucose degradation increased sucrose losses by only 0.03%, but this may vary with factory and region. To summarize, up to the present time, there has been no better marker than glucose reported for the determination of sucrose losses across clarifiers and evaporators, but only minimum losses are given. Furthermore, as factory flow rates fluctuate constantly, large amounts of samples are required in studies of sucrose losses across factory processes to obtain precise averages, and only trends are given.

This paper reports some research highlights and new insights from multiple factory studies (Eggleston and Monge, 2004a; Eggleston and Monge, 2004b; Eggleston and Endres, 2003) of sucrose losses across evaporators and clarifiers in U.S. factories.

EXPERIMENTAL

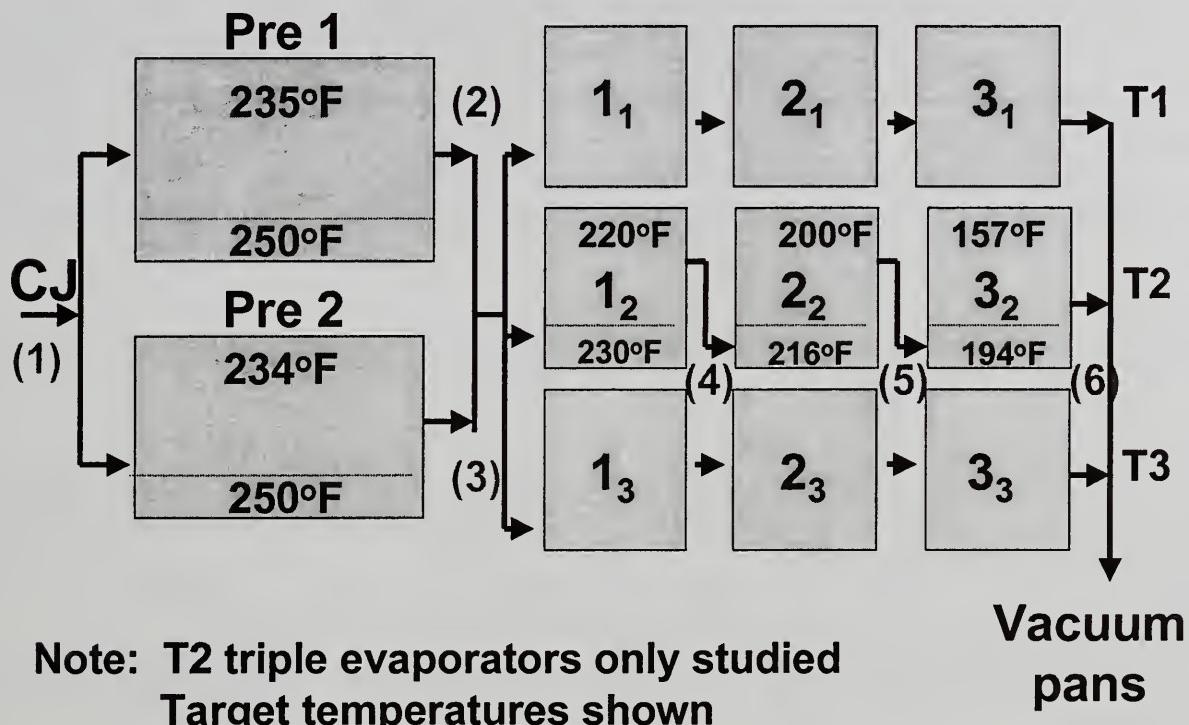
General Sampling Across Evaporators and Clarifiers

Evaporator and clarifier juices and syrups were carefully stored to prevent further chemical degradation reactions and/or microbial growth. For the evaporator studies, each sample was first collected in a large (250 ml) container, and then ~25 ml was immediately poured into a 50 ml container. The two containers were then put in dry ice until transportation to the Southern Regional Research Center in New Orleans, Louisiana, and storage in, a -80°C laboratory freezer, subsequent to laboratory analyses. Glucose, fructose, and sucrose concentrations were measured in juice from the small containers, usually the next day. All other analyses were measured in juice from the large containers. For the clarifier studies, samples collected in one container (250 ml) with sodium azide ($\leq 0.02\%$) added were first frozen on dry ice, and then air-transported from Florida to Louisiana.

Evaporation Studies

Study on Robert's Type Evaporators in Parallel and Series at Cora Texas factory, Louisiana. This study was conducted across the 2001 grinding season, and the season average cane grinding and flowput rates were 13,845 short tons/h and 2105 gallons/min, respectively. The cane processed was 100% green billeted cane, and the factory operated a hot lime clarification process (Eggleston et al, 2003). The factory evaporator station is shown in Figure 1. The two pre-evaporators fed vapors to the triple-effect evaporators, juice heaters, and vacuum pans; there was no bleed off the triple effects. The factory aimed for nine days maximum between cleaning of individual evaporators. Cleaning

occurred by using HCl (2.5-3.5% concentration which was dependent on the evaporator body) for 2h. Evaporator retention times (R_t s) were calculated from the juice volumetric flow rates, evaporator body volumes, and average masses of °Brix in each body according to Honig (1963); assumptions could only be made regarding volumetric flow rates because they change constantly in a factory (Eggleston and Monge, 2004a).



**Note: T2 triple evaporators only studied
Target temperatures shown**

Figure 1. Configuration of Robert's-type evaporators at Cora Texas factory, Louisiana. (From Eggleston and Monge, 2004a,b). Sample points are denoted by numbers in parenthesis.

The effect of three different target final evaporator syrup (FES) pHs were studied in one day, and the factory was flushed out at the target FES pH at least two hours before sampling. Sampling was repeated every 10 min on five consecutive occasions to constitute a sampling period. Each sampling period was repeated three times across the grinding season: early (Oct 2), mid (Nov 6) and late (Dec 11) season. Sometimes the target pH of the FES was not achieved but the pH obtained was taken. Clarified juice, and evaporator juice/syrup entering and exiting each evaporator body were collected taking into account the calculated R_t s (Table 1), and sampling points are shown on Figure 1. To prevent flashing on sampling and for safety reasons, cooling heat exchangers were installed at the sampling points situated at the bottom of the two pre-evaporators and first evaporator body.

Table 1. Calculation^a of Retention Time in Factory Evaporator Bodies Based on an Average Factory Flow Rate of 12,800 Short Tons Cane/Day

	Evaporator Bodies				
	In Parallel ^b		Second Set of Factory Triples in Series ^b		
	Pre-E 1	Pre-E 2	1 st	2 nd	3 rd
Size (ft²)	30,000	25,000	12,500	12,500	12,500
Mean °Brix %	20.1	20.1	28.3	36.8	55.0
Density of juice at mean °Brix	1.081	1.081	1.118	1.159	1.257
Mass of °Brix in the body (Kg)	7026.0	4804.0	4496.0	6060.7	9824.1
Retention time (mins)	11.35^c	9.49^c	9.99^d	13.46^d	21.82

^a Based on Honig (1963) calculations

^b See Figure 1

^c Flow into parallel pre-evaporators was 55% into Pre-E1 and 45% into Pre-E2

^d Flow into the T2 factory triple set of 1st, 2nd, 3rd evaporators was 40% of the total flow imput into the three sets

Because the factory had two pre-evaporators in parallel and three triple effects in series, it was able to stagger the cleaning of individual evaporators over a nine day cleaning cycle. As a consequence on any given day of sampling each evaporator body was at a different time between cleanings, and these were noted.

Evaporator Cleanings were staggered over 9 days:

Day:	1	2	3	4	5	6	7	8	9
Evaporator cleaned:	Pre-E1	Pre-E2	--	Triple 1	--	Triple 2	--	Triple 3	--

Study on Robert's Type Evaporators in Series and a Plate Evaporator at Raceland Factory, Louisiana.

This study was conducted at the latter end of the 2001 grinding season, and the season average cane grinding and flowput rates were 12,105 short tons/h and 1850 gallons/min, respectively. The cane processed was 95% billeted cane (30%green/70%burnt) and the rest was whole-stalk cane (15%green/85% burnt). An average FES pH of 6.11 was operated during this study.

The factory evaporator station is shown in Figure 2. The juice exiting the 2nd Robert's body or effect was a combination of juice circulating in the 2nd body, juice circulating from the 2nd body into the plate evaporator, and juice exiting the plate evaporator being recirculated in the 2nd body. Vapor was bled off the 2nd evaporator to the juice heaters and continuous vacuum pan. Usually, the factory aimed to wash-out its pre-evaporator every 70,000 short tons of cane (~ 5-6 days), and the other evaporators were cleaned together after ~ 9-10 days, although this varied across the season. The plate evaporator was cleaned by boiling caustic soda in the evaporator for approximately 4h. The Robert's type Pre-, 1st and 4th evaporators were cleaned with HCl and ammonium bifluride, and the 2nd and 3rd evaporators with just HCl. R_s in each Robert's type evaporator body were calculated according to Honig (1963), based on a factory flow rate of ~14,000 short tons cane/day. Calculated R_s were 2.9 min in the pre-evaporator, and increased from 2.7 to 8.9 min across the other evaporators; R_t across the plate evaporator (calculated from the av. factory flow rate and total hold-up volume with both steam and juice sides flooded [10,506 L water]) was only 1.1 min.

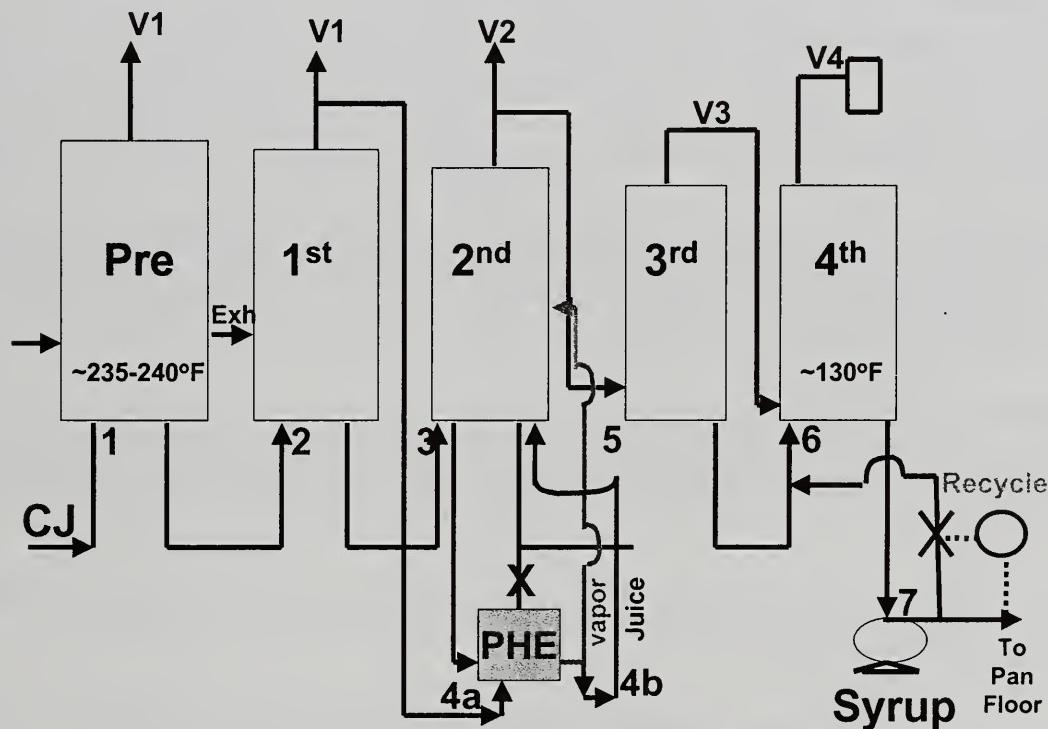


Figure 2. Configuration of Robert's-type and plate evaporators at Raceland Factory, Louisiana. (From Eggleston and Endres, 2004).

Clarified juice and evaporator juice/syrup exiting each evaporator body were collected taking into account the calculated R_s, and sampling points shown on Figure 2. Sampling was repeated five times, every 20 min. Samples were collected on 5 separate days, between November 20 and December 4, covering two adjacent cleaning cycles:

	Days Between Evaporator Clean-outs				
	Nov 20	Nov 23	Nov 27	Nov 29	Dec 4
Pre-, 1 st , 2 nd , 3 rd & 4 th Robert's-type Evaporators	1	5	9	1	6
Plate Evaporator	0 ^a	4	8	1	6

Study on Juice and Syrup Clarifiers at U.S. Sugars Factory, Florida.

Sucrose losses were studied across the juice and syrup clarification processes during the 2001 grinding season. The season average sugarcane grinding rate was 26,000 short tons/day and juice flow to heaters was ~ 4630 gallons/min. The sugarcane processed was 100% burnt billets.

Hot lime clarification was newly operational at the Florida factory. The clarifier studied was a Dorr and the temperature was ~210-220°F (98.9-104.4°C). In hot liming, the pre-heated (~210°F) juice had milk of lime (~12 baume) automatically added into an in-line mixer just before the flash tank, before being flocculated and allowed to settle in the clarifier. Six composite factory samples were taken every 10 min, in and out of the clarifier, taking a 3h R_t (estimation only) into account.

The syrup was clarified by a phosphatation process, and the clarifier temperature was ~180-185°F (82.2-85.0°C). Six composite factory samples were taken every 10 min, in and out of the clarifier, taking a 1h R_t (estimation only) into account.

Sucrose, Fructose and Glucose

The determination of sucrose, fructose and glucose in cane juice by GC was based on the oximation-silylation procedure of ICUMSA method GS7/4-22 (1998) with modifications (Eggleston et al, 2003).

°Brix. The mean °Brix of triplicate samples was measured using an Index Instruments TCR 15-30 temperature controlled refractometer.

% Chloride was measured, at least in triplicate, by potentiometric titration.. Samples (15 ml) with added nitric acid (2M; 2.5 ml) were titrated against silver nitrate (0.1M) using a Metrohm™ 716 auto-titrator, with a silver ring Metrohm™ electrode.

Color and Turbidity were measured as the absorbance at 420 nm and calculated according to the official ICUMSA method GS2/3-9 (1994). Juices and syrups were diluted in triethanolamine /hydrochloric acid buffer (pH 7) and filtered through a 0.45 µm filter. Juice (25 g) was diluted in 75 ml of buffer, and syrup (10 g) was diluted in 90 ml.

pH was measured at room temperature (~ 25 °C), using an Ingold™ combination pH electrode calibrated at room temperature using two different pH buffers (pH 7 and 10). The electrode was connected to a Metrohm 716 DMS pH meter.

Calculation of Evaporation Rate. Percentage evaporation rate was calculated according to the following simple equation:

$$\% \text{ Evaporation Rate} = \frac{\text{Av. } ^\circ\text{Brix}_{\text{OUT}} - \text{Av. } ^\circ\text{Brix}_{\text{IN}}}{\text{Av. } ^\circ\text{Brix}_{\text{OUT}}} \times \frac{100}{1}$$

RESULTS AND DISCUSSION

Sucrose Losses Across Evaporators

Effect of Scaling on Sucrose Losses Across Robert's-type Evaporators in Parallel and Series.

The most frequently used method in the U.S. to monitor evaporation performance is to simply measure daily $^\circ\text{Brix}$ changes for individual evaporator bodies, although monitoring the evaporation rate is more accurate. From a comprehensive evaporator study (Eggleston and Monge, 2004a,b) conducted at Cora Texas, Louisiana, factory, typical average $^\circ\text{Brix}$ across a cleaning cycle are shown in Figure 3. The $^\circ\text{Brix}$ decreased markedly in the later triple evaporators 6 days after the last clean (Figure 3). This is because with increasing time after a clean, scale builds up in evaporators that causes a reduction in the heat-transfer coefficient and, therefore, less evaporation occurs and $^\circ\text{Brix}$ decreases. In this factory, most evaporation occurred in the last evaporator to minimize sucrose losses, as the lowest temperatures and highest $^\circ\text{Brix}$ s occur there. Some factories in other parts of the world (Henrique Amorim, personal communication) allow most evaporation to occur at the front end of the evaporator station in order to create more vapor bleed for heating juices. However, the consequences will be higher sucrose losses, which may be more costly than vapor bleeding.

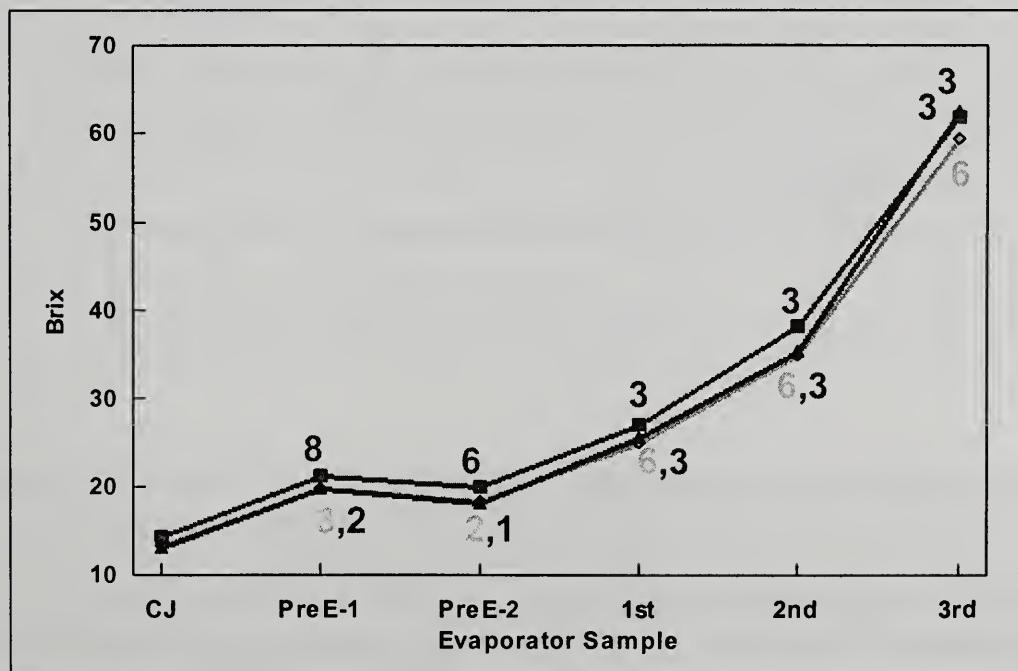


Figure 3. Effect of time between clean-outs on $^\circ\text{Brix}$ levels across an evaporation station. Numbers on the graph denote days after the last clean-out. (From Eggleston and Monge, 2004a).

The typical effects of scaling on sucrose losses are illustrated in Figure 4 using histograms of glucose changes across individual evaporator bodies. Glucose formation, indicating that sucrose had inverted, is depicted by histogram bars above the zero line. Glucose reduction is depicted by bars below the zero line and can only occur if glucose is either degraded or precipitated out. Most inversion occurred in the pre-evaporators (Figure 4) because of the higher temperature, larger heating surfaces, and lower °Brix. Less sucrose losses occurred in the second pre-evaporator (Pre E2) than the first (Pre E1) even though they existed in parallel (Figure 1), which was because the Pre E2 was smaller ($25,000\text{ft}^2$) than the Pre E1 ($30,000\text{ft}^2$) (Purchase et al, 1987). As can be seen in Figure 4, scaling had a dramatic effect on increasing sucrose losses (Eggleston and Monge, 2004a). For both pre-evaporators, as the time between wash-outs increased from 1 to 8 days, the amount of sucrose inversion increased proportionally (Figure 4). This can be explained by scale formation that reduces the heat-transfer coefficient, which in turn raises the compensatory temperature and retention time in the evaporator (Eggleston and Monge, 2004a). Furthermore, when the triples were just 3 days after the last clean-out (Figure 4) no sucrose inversion was detected; however, after 6 days some inversion was detected in the 1st and 2nd bodies.

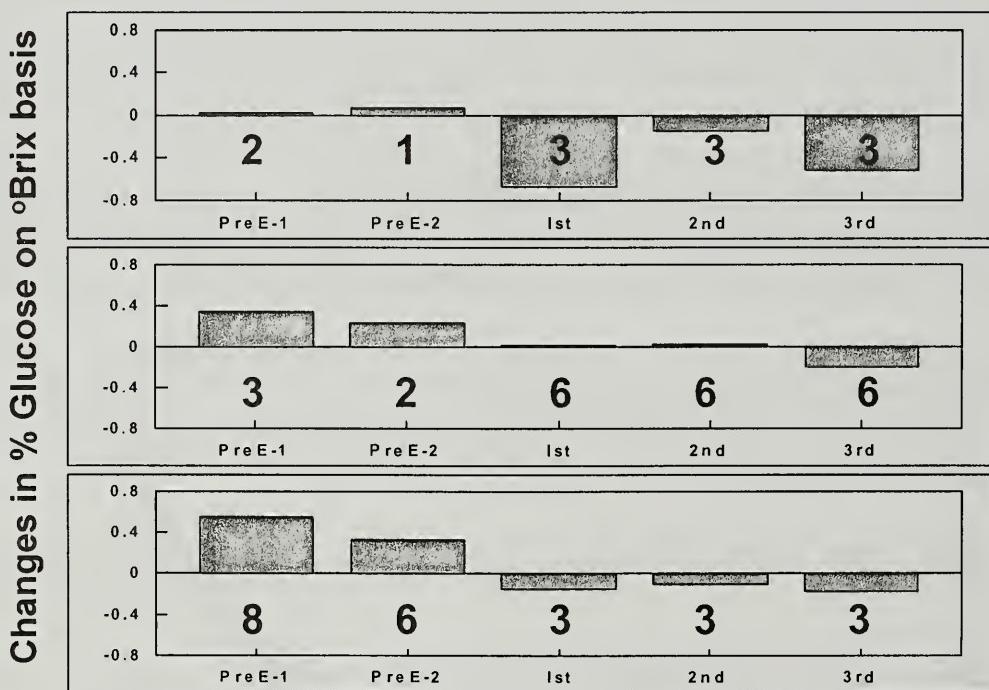


Figure 4. Dramatic effect of scaling on increasing sucrose losses. Numbers on the histograms denote days after last clean-out. (From Eggleston and Monge, 2004a).

Effect of Varying the Target Final Evaporator Syrup pH on Sucrose Losses.

For comparison purposes in this study, sample pHs were measured at room temperature. However, at the higher processing temperatures the pHs are always lower because the dissociation of water and sugars provides more H⁺ ions. It is known that pH usually drops across evaporator stations because of: (1) the precipitation of basic salts, (2) the formation of acids from sugar degradation reactions, (3) the increasing °Brix concentrations concentrating H⁺ ions, and (4) the release of small quantities of ammonia from amino compounds. To remove the strong effect of °Brix concentration, we

measured the pH of each sample at the °Brix of the associated clarified juice. The typical effect of adjusting the °Brix of the sample on pH is shown in Figure 5. As expected, the °Brix adjusted pH was higher than the non-adjusted sample pH, although this difference usually decreased across the station up to the second body, suggesting that other contributors to the pH decline, i.e., precipitation of basic salts, other than the °Brix concentration effect, became continuously more important.

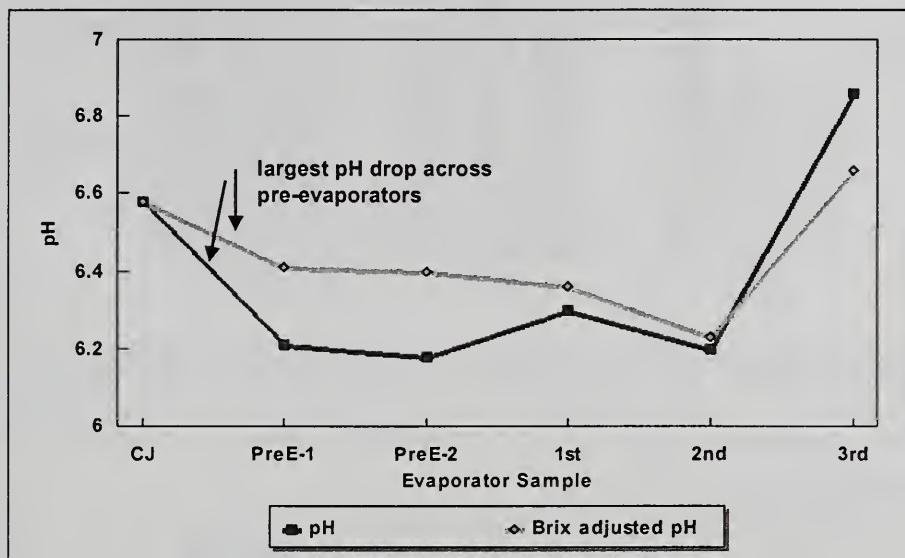


Figure 5. Typical effect of adjusting evaporator station samples to the clarified juice °Brix on pH at 25°C. (From Eggleston and Monge, 2004b).

A surprising but very consistent phenomenon occurred in the 3rd and last evaporator (Figures 5 and 6). The pH dramatically increased and, as can be seen in Figure 5, this was not because of °Brix concentration effects. Measurement of condensate pHs from this body often showed a marked decrease (Eggleston and Monge, 2004b) which strongly indicates that this pH increase in the last body is because of the evaporation of volatile acids. Day (2002) in the same grinding season at another Louisiana factory reported that the concentration of volatile acids (formic and acetic) was greater in the condensate of the last evaporator body, and caused major pipe corrosion problems. The microbial quality of sugarcane wash water was stated by Day (2002) to be the major source of these acids. This phenomenon (Figure 5) has not been reported before in other geographical areas, which may be because the presence of volatile acids is lower, or a more simpler explanation is that it has not been studied.

Effect of Different Target Final Evaporator Syrup pHs on Evaporator Station pH Profiles.

The effect of changing the target pH of the FES on the evaporator station pH profiles across the season are shown in Figure 6. The major factor affecting the pH of the final evaporator syrup was the pH of the clarified juice. The phenomenon of the dramatic pH increase in the last body was consistent across the whole season. The largest drops in pH occurred in the pre-evaporators (Figures 5 and 6), indicating that the most inversion occurred there.

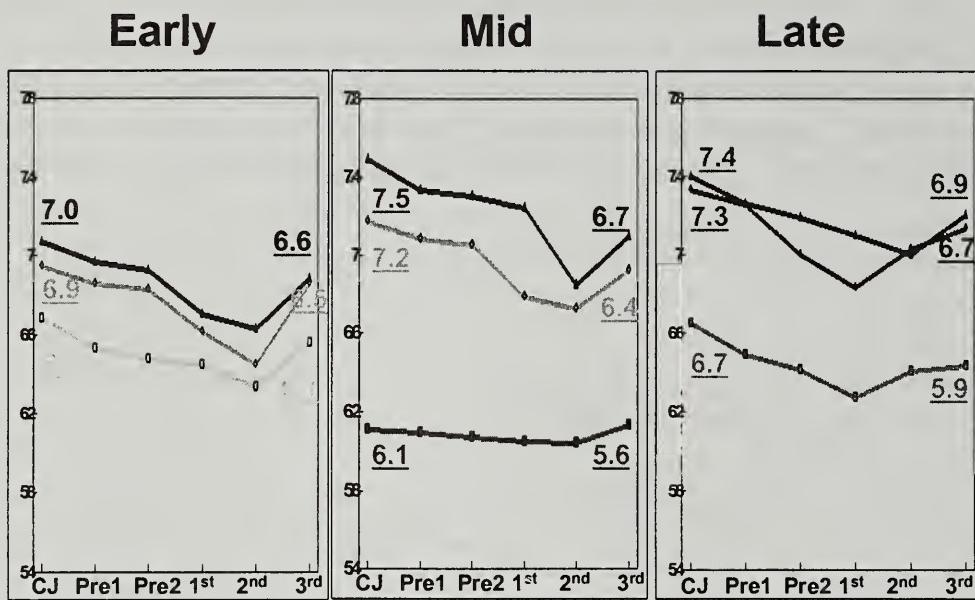


Figure 6. Effect of changing the target FES pH on seasonal changes in °Brix adjusted pH profiles.^a (From Eggleston and Monge, 2004b).

^a Unadjusted CJ and FES (3rd body) pHs are underlined)

Target pH and Seasonal Effects on Glucose Concentrations in Clarified Juices.

The effect of changing the target pH of the FES on the average glucose concentrations in clarified juices (CJs) are illustrated in Figure 7. On a given sampling day, as expected, glucose decreased with an increase in target pH because of alkaline degradation. It can also be seen from Figure 7 that the CJ glucose decreased across the season irrespective of the target FES pH, and can be attributed to the quality of the sugarcane supply. The quality of the sugarcane supply in Louisiana is lowest in early season (Eggleston, 2000a,b), with the highest levels of glucose occurring because of immature cane that contains more invertase and, therefore, more invert. This has important implications on the extent of sucrose losses which occur, as glucose as well as pH can catalyze or further induce the inversion of sucrose at high temperatures (Richard, 1988).

Effects of pH on Sucrose Losses.

Typical effects of target FES pHs from 5.6 to 6.7 are shown in Figure 8. It is not recommended that factories target FES pHs at 5.6 because of high inversion, but this sometimes happens when problems occur (Eggleston, 2000a). Before this work in 2001, typical FES pHs at Louisiana factories were 5.8-6.0 (Eggleston 2000a). As the FES target pH increased from pH 5.6 to 6.7, inversion in the pre-evaporators usually decreased markedly. Higher inversion in PreE1 was most likely because it was less clean, i.e., 5 days after the last clean (Figure 8) compared to the PreE2 which had been cleaned 2 days before. By FES pH 6.7, sucrose inversion was relatively low in PreE1 and not even detected in PreE2 (Figure 8). The only other evaporator where inversion was detected was in the 2nd. Seven days had elapsed after the last cleaning of the 1st, 2nd, and 3rd evaporators, and the scale build-up caused inversion to occur because of increased retention times and higher compensatory temperatures. However, glucose formation and consequently sucrose losses still occurred in the 2nd body at FES pH 6.7 and, therefore, scaling effects can override pH effects.

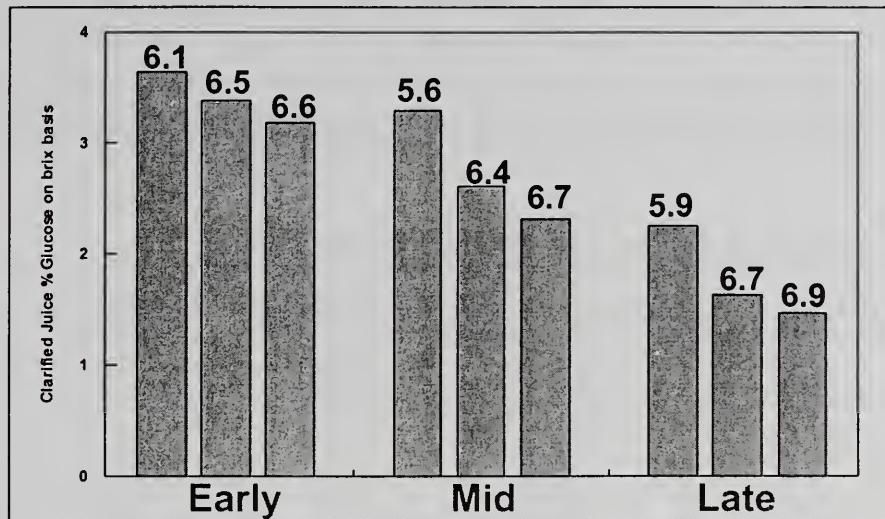


Figure 7. Effect of target FES pH on glucose in clarified juices across the season. Numbers on the histogram bars denote the average FES pH. (From Eggleston and Monge, 2004b).

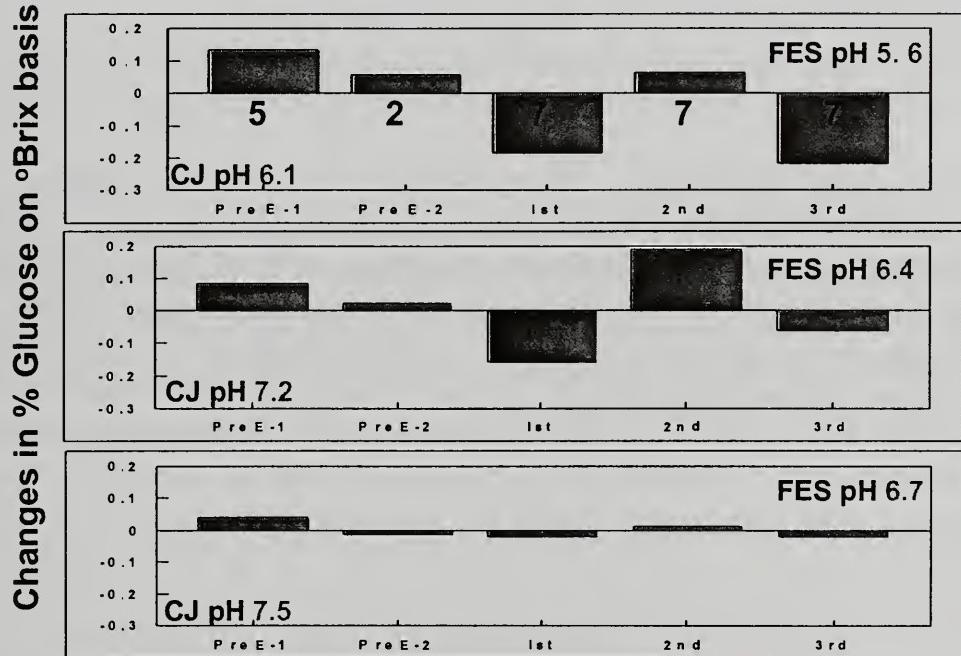


Figure 8. Effect of changing the target FES pH on sucrose losses. Mid season data shown. Numbers on the histogram bars denote days after the last clean-out. (From Eggleston and Monge, 2004b).

Sucrose Loss Calculations.

The effect of changing the target FES pH on % sucrose losses are shown in Table 2. As expected, the lower the pH, the higher the losses and *vice versa*. Although a FES target pH of 5.6 does not occur in factories because of expected high inversion, it sometimes occurs when deteriorated cane is being processed or factory problems occur. In 2000 many Louisiana factories had a target CJ pH of ~ 6.6 to give a FES pH of ~ 6.0, although pHs of 5.9 in the FES often occurred (Eggleston, 2000a). Results in Table 2 show that by increasing the target FES pH to at least pH 6.3 causes a

marked decrease in losses, particularly in mid and late season. Although the least inversion occurred at FES pH 6.7, this is not recommended because too much lime would be consumed and the potential for more scale formation from calcium salts increased, which could in turn cause more inversion. Overall, a compromise target FES pH range of 6.3-6.5 is recommended to reduce sucrose losses.

Table 2. Effect of target FES pH on calculated % sucrose losses^a

Target pH		% Sucrose Losses ^{b,c}			
FES	CJ	Early	Mid	Late	Season Av.
5.6	~6.1-6.3	0.810	0.307	0.855	0.657
5.9	~6.7	0.690	0.276	0.669	0.545
6.3	~7.1	0.531	0.235	0.590	0.452
6.7	~7.4	0.370	0.075	0.370	0.272

^a Only conservative (minimum) estimates

^b Based on the formula of Schaffler et al (1985), on a °Brix basis

^c Some % loss data were obtained as extrapolations from graphed data

Seasonal effects of sucrose losses across evaporators were also dramatic with sucrose losses being lower in mid-season when the best sugarcane quality occurs (Eggleston, 2000a). The higher losses in the early season can be attributed mostly to the low quality of cane (Figure 7), as the evaporators were comprehensively cleaned in the off-season before grinding began. Greater amounts of impurities, including glucose (Figure 4), fructose, acids, and salts would have further catalyzed inversion of sucrose. Higher target pHs in the early season or when immature cane is being processed, may offset these extra losses, although the target pH would have to be a compromise as increased lime addition will counteractively increase some scaling. Some of the highest sucrose losses also occurred in the late season (Table 2). Although cane quality often decreases again (Eggleston 2000a,b) in the late season (but not to the extent of the early season), these increased losses are most likely because of the build-up of scale resistant to cleaning in the later bodies after the pre-evaporators, which becomes worse as the season endures (Eggleston and Monge, 2004b). This is further evidenced by the considerable improvement of overall losses in late season, if the losses in the later evaporator bodies were removed. This resilient scale most likely contains silicate which is the most difficult to remove scale, and which is more predominant in the later bodies (Godshall and Wartelle, 2002). A higher target pH in late season is not advocated as resilient scale in the later bodies (Figure 8) would override pH effects anyway.

Effect of Target pHs on Minimum Economic Costs.

The minimum amount of U.S. dollars that are lost because of the sub-optimization of target FES or CJ pHs, was calculated according to the following equations:

$$(CG \times [E/100] \times [CJP/100]) \times L/100 = S \quad \text{Eqn. (1)}$$

then

$$([S/RSS \times 100] \times 2000) \times RSP = \text{U. S. dollars lost per season} \quad \text{Eqn (2)}$$

Where:

CG = Short tons of sugarcane ground for the whole grinding season

E = Extraction of juice%cane

CJP = Clarified juice % sucrose (season av. based on pol)

L = Sucrose loss (%)

S = Short tons of sucrose lost

RSS = Raw sugar % sucrose (season av. based on pol)

RSP = Price per lb of raw sugar in U.S. dollars in 2001 (i.e., 20 cents per lb)

The calculated results are shown in Table 3. Even though dollar losses are only minimal and conservative, considerable costs were still incurred by the factory because of unwanted sucrose losses across the evaporators. Although much less profit losses occurred at FES pH 6.7, this target pH it is still not recommended. A compromise in target CJ or FES pH has to be achieved by any factory to ensure no excessive lime addition occurs and, even worse, more calcium scale and color formation.

Table 3. Effect of target FES pH on minimum economic costs of season sucrose losses across evaporators.

Target FES pH	Target CJ pH	Av. lbs raw sugar lost/ton cane	Min. U.S. \$ losses across season
5.6	~6.1-6.3	1.84	\$470,629
5.9	~6.7	1.52	\$390,400
6.3	~7.1	1.26	\$323,781
6.7	~7.4	0.76	\$194,842

Effects of Scale on Sucrose Losses Across Robert's-type Evaporators in Series and a Plate Evaporator.

At Raceland raw sugar factory in Louisiana, for the last 34 days of the 2001 grinding season the first installation in the U.S. of a full-scale plate evaporator (Alfa Laval EC 700TM) was operational. The plate evaporator was operated as a booster to the 2nd evaporator of an evaporator station made up of five Robert's-type calandria evaporators in series (Figure 2). Some measured improvements from the plate evaporator (Swift et al, 2002) included 1) an increase in the grinding rate (on average by 1500 tons cane/day), 2) improved steam economy by up to 130lbs steam/ton cane, 3) imbibition to mill increased by 10-15% which improved extraction, and 4) a small R_t of 1.1min.

$^{\circ}$ Brix increase) across the plate evaporator was ~11.5%. Over the two adjacent cleaning cycles studied, scaling had a marked effect on reducing $^{\circ}$ Brix and % evaporation values in the plate and later Robert's bodies (Figure 9). In the first cleaning cycle, scaling effects became most apparent between 4-9 days after the last clean, particularly in the last body. In the second cleaning cycle, the scaling effects became worse. This indicates that the plate and Robert's type evaporators became more susceptible to scaling and/or cleaning problems with prolonged use.

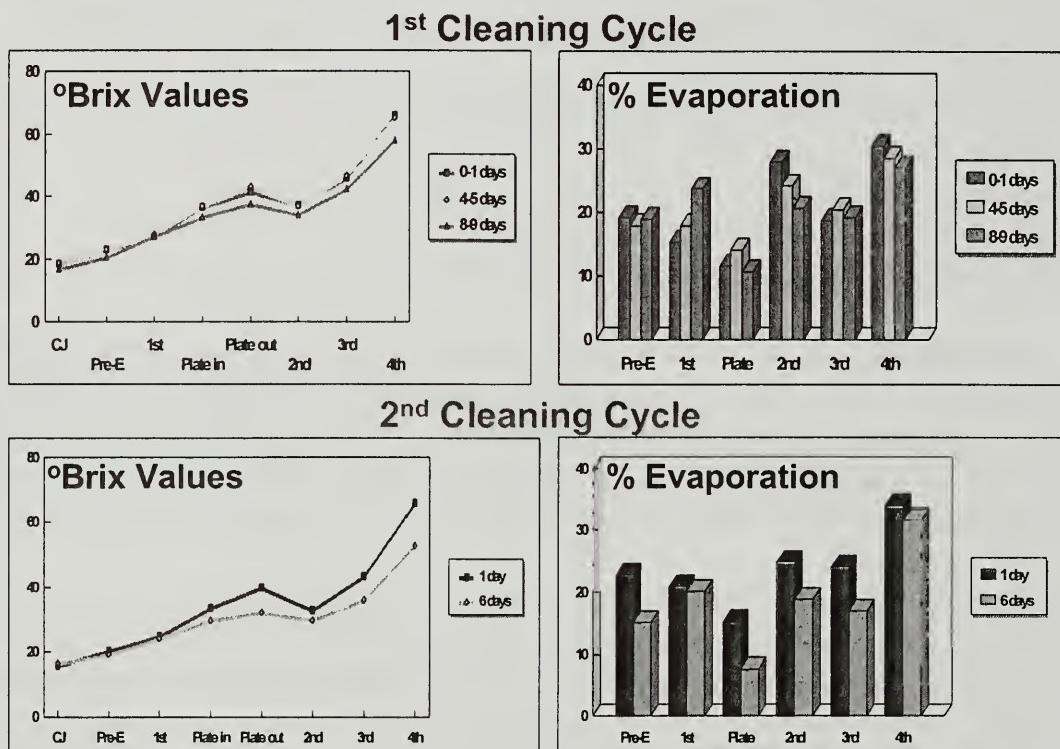


Figure
scaling (days after last clean-out) on evaporation performance across two cleaning cycles. (From Eggleston and Endres, 2004).

9. Effect of

The effect of scaling on sucrose losses is also illustrated in Figure 10. Similar to Cora Texas factory in Louisiana, most sucrose inversion losses occurred in the pre-evaporator because of the higher temperature, larger heating surface, and lower $^{\circ}$ Brix. In the first cleaning cycle studied, when the evaporators were clean (0-1 days after the last clean) inversion was detected mainly in the pre-evaporator, with trace inversion in the 4th body. Even after 4-5 days after cleaning, no inversion was detected across the plate evaporator, but inversion had markedly increased in the 2nd and 4th evaporators. However, by 8-9 days after cleaning, sucrose inversion had occurred across the plate evaporator and, except the 3rd, in all the Robert's evaporators. This was more dramatic in the second cleaning cycle where, 6 days after cleaning, marked inversion occurred across all the evaporators except the 3rd, and the plate susceptibility to scaling and inversion became worse with prolonged use of the plate. The sucrose losses consistently measured in the last evaporator at this factory, even just after a clean, was a phenomenon that was not observed at Cora Texas factory and highlights the differences between factories.

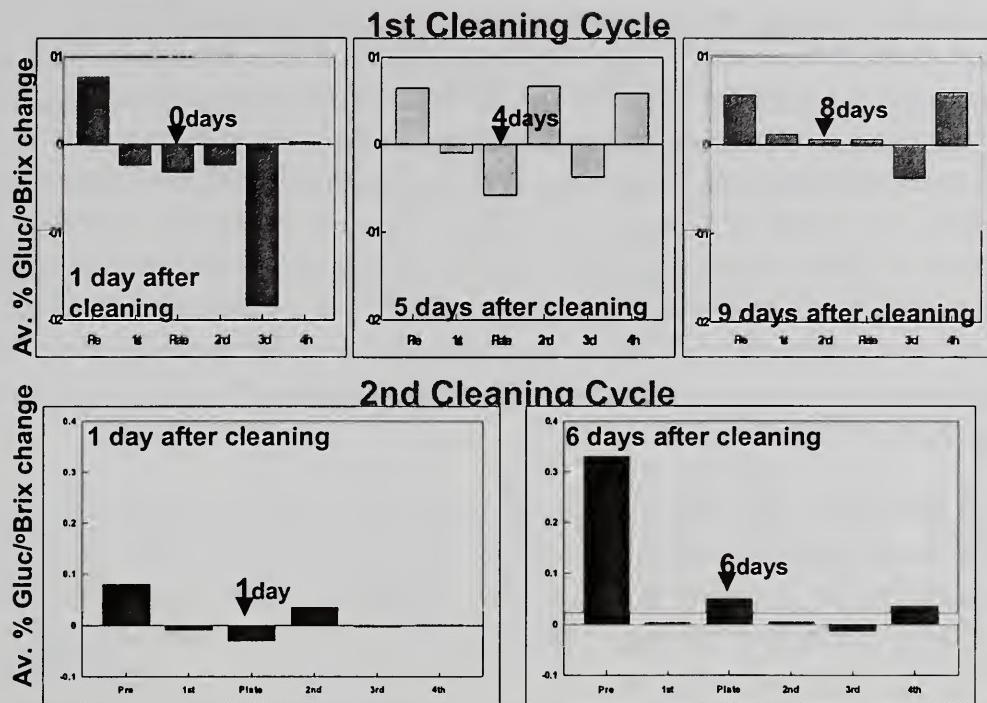


Figure 10. Effect of scaling (days after last clean-out) on sucrose losses. (From Eggleston and Endres, 2004).

Calculated Minimum Economic Costs Across Robert's-type Evaporators in Series and a Plate Evaporator.

The minimum economic cost of sucrose losses, calculated according to the two equations stated above, are listed in Table 4. As limited samples were taken, these results can only be taken as very conservative approximations. Considerable profits were lost across the pre-evaporator, and although the plate was susceptible to sucrose losses because of scaling, the low R_t kept profit losses relatively lower.

Table 4. Minimum economic costs of season sucrose losses across Robert's-type evaporators in series and a plate evaporator.

Evaporator	% Sucrose Loss ^a	Lbs sucrose lost/ton cane	Approx. U.S. dollars season losses
Pre	0.216	0.615	\$ 112, 395
1 st	0.014	0.040	\$7,285
Plate	0.011	0.032	\$5,932
2 nd	0.045	0.128	\$23,416
3 rd	0.000	0.000	\$ 0
4 th	0.077	0.218	\$39,910

^a Based on the formula of Schaffler et al (1985), on a °Brix basis

Sucrose Losses Across the Juice and Syrup Clarifier of a Florida Factory.

The Florida factory studied had both juice and syrup clarification unit processes. This was because they had a refinery next to the factory that did not utilize a clarification process and, therefore, the turbidity of the syrup needed to be lower than usual. The syrup clarification process was completely different from the juice (hot lime) clarification process, being based on flocculation-phosphatation. In this limited study, we found an average % turbidity removal across the juice clarifier of only 78.8%. Eggleston et al (2003) found turbidity removal usually ~96 % for hot liming in Louisiana. Furthermore, the turbidity of the clarified juice was ~5072 ICU, which is high, (Eggleston et al, 2003) and highlights the need for the syrup clarification step. Average turbidity removal across the syrup clarification step was 72.6%, and the clarified syrup turbidity was 1144 ICU, i.e., suitable for the refinery (Eggleston et al, 2003).

Average chemical parameter measurements and changes across both the juice and syrup clarifiers are shown in Table 5. The pH values across the juice clarification process were extremely high, and indicates over-liming. Since this study, the factory has markedly decreased its target pHs. Too high a pH means excessive consumption of lime, evaporator scaling and color formation from the alkaline degradation of invert. Alternatively, too low a pH means excessive sucrose losses. Within experimental error, °Brix and chloride did not change across the juice clarifier. The +0.08% change in sucrose highlights the difficulty of measuring sucrose losses using sucrose changes. In contrast, the positive increase in glucose indicates sucrose inversion did occur. The negative change in fructose compared to glucose highlights the higher reactivity of fructose and why it cannot be used to measure inversion losses. The pH values across the syrup clarifier were much lower (Table 5) than for the juice clarifier.

°Brix decreased by -2.58% points which can indicate some sucrose breakdown to volatile compounds, but is more attributable to the addition of milk of lime and water to remove the scum from the phosphatation process. This dilution effect also explains the reduction in chloride (Table 5). The positive change in %sucrose across the syrup clarifier, further confirms that it cannot be used to measure losses, which did occur as both the glucose and, to a lesser extent, fructose values increased markedly (Table 5).

In the juice clarifier, within experimental error, the % changes in chloride (+0.002%) and °Brix (+0.05%) were the same. Furthermore, as can be seen on Figure 11a, °Brix tended to trend with chloride, indicating both parameters can be used to as reference markers to measure sucrose losses using Schaffler et al (1985) equations. In strong contrast, in the syrup clarifier, there was no trend between the % chloride and °Brix changes (Figure 11b), and °Brix was more preferentially destroyed to chloride (Table 4), strongly suggesting chloride would be a better reference marker to calculate sucrose losses. This was confirmed, when the sucrose losses were calculated (Table 6).

Table 5. Average chemical parameter measurements across juice and syrup clarifiers.

Chemical Parameter	Average \pm SD		Δ In to Out
	In	Out	
Juice Clarifier			
pH (room temp)	9.38 \pm 0.28	8.46 \pm 0.08	-0.92
°Brix (%)	12.93 \pm 0.75	12.7 \pm 0.21	-0.05
Sucrose (%)	11.91 \pm 0.64	11.99 \pm 0.15	+0.08
Glucose (%)	0.123 \pm 0.02	0.129 \pm 0.008	+0.006
Fructose (%)	0.138 \pm 0.02	0.135 \pm 0.007	-0.003
Chloride (%)	0.074 \pm 0.002	0.076 \pm 0.00	+0.002
Syrup Clarifier			
pH (room temp)	7.31 \pm 0.07	6.84 \pm 0.29	-0.47
°Brix (%)	66.95 \pm 1.10	64.37 \pm 0.29	-2.58
Sucrose (%)	12.72 \pm 0.30	13.08 \pm 0.32	+0.36
Glucose (%)	0.169 \pm 0.006	0.216 \pm 0.014	+0.047
Fructose (%)	0.172 \pm 0.01	0.203 \pm 0.01	+0.031
Chloride (%)	0.352 \pm 0.007	0.341 \pm 0.004	-0.011

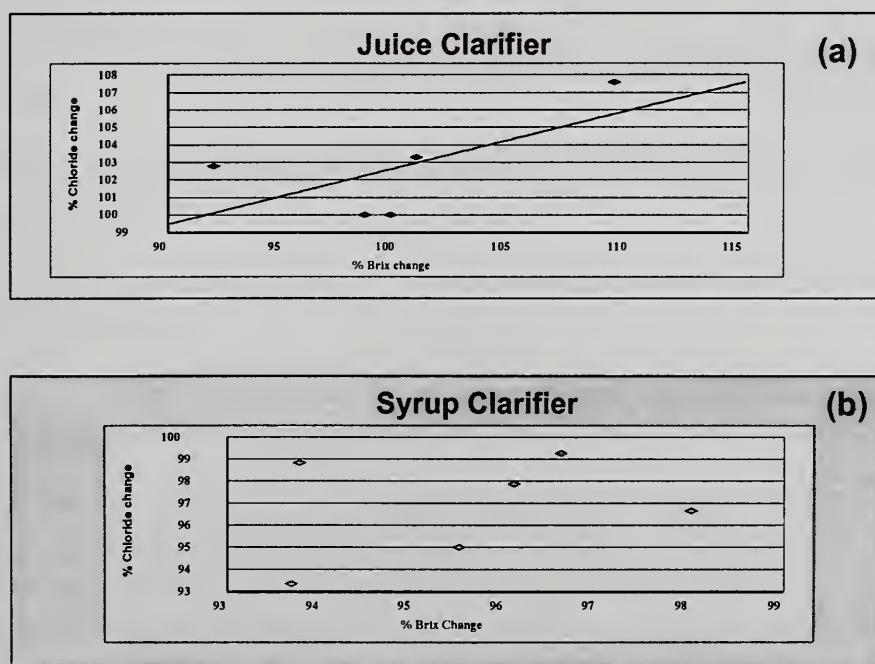


Figure 11. % °Brix (refractometric) changes versus % chloride changes.

Across syrup clarification where sucrose losses were high, ranging from 0.57-1.26%, °Brix markedly underestimated sucrose losses compared to % chloride. In the case of juice clarification (Table 6), no sucrose losses were detected in three of the samples because of the extremely high operating pH. When sucrose losses were detected, there was generally small differences between using °Brix or chloride as a reference (Table 5). The variation in sucrose losses across the juice clarifier (range: 0.00 - 0.58%) may be attributable to highly fluctuating mixed juice flow rates reported at this factory. Overall, this research highlights the need for preliminary studies to ascertain if °Brix is suitable as a reference marker to measure sucrose losses across the unit process of study.

Table 6. Calculated % sucrose losses.

Composite Juice Number	% Sucrose Losses Across Juice Clarifier	
	Gluc%Sucrose on °Brix Basis	Gluc%Sucrose on % Chloride Basis
1	0.00	0.00
2	0.00	0.00
3	0.00	0.00
4	0.0061	0.00
5	0.47	0.417
6	0.58	0.345

Composite Syrup Number	% Sucrose Losses Across Syrup Clarifier	
	Gluc%Sucrose on °Brix Basis	Gluc%Sucrose on % Chloride Basis
1	0.54	0.70
2	0.64	0.85
3	0.54	0.64
4	1.01	1.26
5	0.53	0.77
6	0.50	0.57

Obviously, the operating pH of the two different clarification processes governed the extent of acid inversion of sucrose that occurred. Although the high pH conditions across the juice clarifier reduced sucrose losses (see Tables 5 and 6), over-liming would exacerbate scaling in the evaporators and increase color formation (results not shown) from the alkaline degradation of glucose and fructose. In contrast, the lower pH conditions across the syrup clarifier minimized color formation (results not shown), but greatly increased sucrose losses (Table 6). In U.S. factories, color is not a major concern because seldom is there a penalty for color in raw sugar from the refinery, whereas sucrose losses are a great concern because of the expensive loss in yield profits.

RECOMMENDATIONS

- * Control of the Final Evaporator Syrup (FES) pH is recommended to minimize sucrose losses in the clarifiers and evaporators. A target FES pH of 6.3-6.5 (equivalent to a target clarifier juice pH of ~7.0-7.2), as measured at room temperature, is recommended.
- * Target FES and CJ pHs need to be higher in early season or when immature cane is being processed, to reduce excessive sucrose losses (compromise).
- * Predictor equations of evaporator sucrose losses currently being used in the sugar industry (for example, Vukov 1965), need to incorporate a scaling component to improve estimations.
- * Preliminary studies are recommended to ascertain if °Brix is suitable as a reference marker to measure sucrose losses across the unit process of study.
- * Future studies of sucrose losses across evaporator stations, need to take into account the cleaning status of the individual evaporators.
- * Further studies on scale reducers or inhibitors are warranted.
- * Factory studies are needed on the use of alternative low retention, high heat transfer Plate, Kestner (rising film) or falling film evaporators, particularly as pre-evaporators.

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OPTIMIZATION OF SUGARCANE FACTORY APPLICATION OF COMMERCIAL DEXTRANASES IN THE U.S.

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ABSTRACT

The application of commercial dextranases to break down dextran in U.S. sugar manufacture is still not optimized, partly because of misinformation about where to add the enzyme and which enzyme to use. Furthermore, there is no uniform method to measure the activity of commercial dextranases by producers/vendors/distributors, which has meant that direct comparison of activities is not possible. In this study, a simple titration method to determine the relative activity of dextranases was identified and modified for easy factory use. All activities were confirmed with an accurate IC-IPAD method using a NaOH/NaOAc gradient. Most commercial dextranase enzymes in the U.S. are from a fungal source: *Chaetomium gracile* or *erraticum*, and are available in “non-concentrated” or “concentrated” forms. An approximate 8-10 fold difference in activity exists between the two concentration forms, and activity variations exist within each form. In 2002/03 only “non-concentrated” dextranases were applied in Louisiana to either last evaporator bodies (usually ≤ 10 ppm/syrup) or juice. “Non-concentrated” and “concentrated” dextranases studied at juice pH 5.4-5.8, showed similar maximum activity at 48.9°C or 120°F, as monitored by IC. Dextranase activities, in last evaporator syrup temperature (~63°C or 145°F) and Brix (~65°) conditions, were dramatically reduced (activity began to decrease after 25-30°Brix). Overall, juice applications were more efficient and economical than adding them to evaporator syrups. Application of “non-concentrated” dextranase to evaporator syrup was uneconomical. However, “concentrated” dextranase can be applied to syrup at levels as low as 10 ppm/solids (equiv. to 45 ppm/juice) to remove up to ~37% dextran which is useful to consider when severe dextran problems occur. Heating juice to 48.9°C in the presence of all dextranases, dramatically removed more dextran (3380 ppm/Brix) from a juice than at the current ambient temperature of application (32.2°C or 90°F) and was much more economical. For a “non-concentrated” dextranase, after 10 min at 10 ppm/juice and 48.9°C, ~46.3% dextran was removed compared to 13.6% at 32.2°C. For the “concentrated” dextranase, after only 10 min at only 4 ppm/juice, 66.6% dextran was removed at 48.9°C and was considered an overdose,

compared to 29.6% at 32.2°C. Dextranase was shown to work in the presence of dithiocarbamate biocide in juice, and factory studies are being undertaken to check that no adverse dextran formation is occurring at 48.9°C. Under factory storage conditions, over a grinding season (90 days), the activity of “concentrated” dextranase decreased only slightly (~9%), whereas “non-concentrated” dextranase activity had approximately halved (~46%), and even reduced in activity when stored under refrigeration.

INTRODUCTION

The major contributor to sugarcane deterioration in the U.S., particularly Louisiana, where humid conditions prevail, is from *Leuconostoc* lactic acid bacterial infections. Such infections mostly occur after severe freezes, when cut-to-crush times are delayed, and are also impacted by the harvesting method (Eggleston and Grisham, 2003) and because of poor mill hygiene. *Leuconostoc* species (and to a lesser extent some *Lactobacillus* species) produce an extracellular exo-enzyme dextranucrase which catalyzes the production of dextran from sucrose. Dextran is a glucose polysaccharide comprising mainly α -(1→6) linkages, but also contains a small amount of α -(1→4), α -(1→3) and some α -(1→2) linkages (Robyt and Eklund, 1982). Most dextrans in the sugar industry are linear, but some branching may occur (Edye et al, 1995). Moderate and severe dextran (>1000 ppm/ $^{\circ}$ Brix in mixed juice) in the factory has long been acknowledged as an interrupter of normal processing operations. Formation of dextran not only causes expensive sucrose losses, but the high viscosity associated with this polysaccharide (especially the high MW portion) often slows evaporator and crystallization rates, raises losses of sucrose to molasses, and distorts factory pol readings. Worse still, the factory is penalized by refineries on dextran in the raw sugar. Although clarification processes remove some dextran (Eggleston et al, 2003), commercial dextranase has been used in sugarcane factories to break down dextran, by hydrolyzing α -(1→6) linkages endogenously into smaller, more manageable molecules, and has been used in sugarbeet processing (DeBruijn, 2002). In some Louisiana factories dextran concentrations \geq 800 ppm/ $^{\circ}$ Brix in mixed juice cause the staff to add dextranase, whilst other factories just add it when factory processes are obviously suffering.

The application of dextranases in the sugar industry was pioneered in Australia in the 1970s, and the comprehensive review of this work by Inkerman (1980) is recommended. Dextranase activity is governed by the pH, temperature, residence time (R_s), agitation, substrate concentration, type and concentration of enzyme applied, and initial amount of dextran present. Usually, the higher the dextran concentration the more hydrolysis of dextran occurs (Fulcher and Inkerman, 1976). The use of dextranase is a routine procedure in Australian raw sugar factories, when dextran levels are high and detrimentally affect processing. The properties of commercially available dextranases governed the selection of the addition point in the factory (Inkerman, 1980) and dextranase is usually added in holding juice tanks just before clarification, with minimum R_s s of 15 min and temps of 55-60°C. Australians have not advocated addition of dextranases in high $^{\circ}$ Brix syrup evaporators or tanks because dextranases are not as stable at high syrup temperatures as amylases that are routinely used for starch break down in syrup, or at unfavorable syrup pHs (>6.0), and the $^{\circ}$ Brix has a marked inhibitory effect (Inkerman, 1980). In comparison, in South Africa where diffusers are used, dextranase application in diffuser cane juices was deemed unsuitable mostly because of the high temperatures (Morel du Boil and Wienese, 2002). Consequently, application of dextranases to

evaporator syrups has been advocated (Morel du Boil and Wienese, 2002) but over a 30 min R_t that is not usually available in U.S. factories.

Some U.S. dextranase studies have occurred since the late 1970s. Polack and Birkett (1978) indicated the possibility of using dextranase in plant trials. DeStefano (1988), using laboratory trials, compared the application of several U.S. commercial dextransases for use in mixed juice, final evaporators and syrup storage. DeStefano (1988) advocated the addition of dextranase in syrup storage tanks, as the volumes of material to be treated were smaller and some clean-up had already been achieved via clarification. However, it was acknowledged (DeStefano, 1988) that the pH and °Brix of the syrup are not optimum and relatively higher levels of dextranase would have to be added compared to juice. In the mid 1990s, Edye et al (1997) conducted factory trials of a dextranase from the fungus *Cheatomium gracile* that was stated to be more temperature stable. Cuddihy and Day (1999) have discussed some of the financial implications associated with dextranase treatment. However, the problem with these few U.S. dextranase studies is that they never stated the activity of the enzymes they were applying, and this has led to enormous confusion concerning the appropriate concentration of each commercial dextranase to add. Furthermore, there is no uniform or factory usable method for dextranase activity in the sugar industry.

Since 1996 many Louisiana factories have been utilizing dextranase, but the point of application is extremely varied and reports from some factories have indicated that dextran degradation is very limited and the economical use of the enzymes has been questioned. Furthermore, our initial factory studies of current dextranase applications (20 ppm/solids) in last evaporator syrups (65.5°C; $R_t=20$ min) indicated there were no differences in viscosity changes compared to the 0 ppm control. This study was, therefore, undertaken at the request of the Louisiana raw sugar manufacturing industry to optimize the addition and conditions of dextranase in U.S. factories.

EXPERIMENTAL

Commercial Dextransases

A variety of commercial dextransases currently in use in Louisiana and Florida sugarcane factories, or available in the U.S., were studied and are listed in Table 1. Because the United States Department of Agriculture does not endorse one brand over others of a similar nature, the enzymes have been denoted with numbers. Commercial dextranase 1 was a gift from Dr. Ron DeStefano who had stored it at 4°C. Dextranase 2 was donated by the suppliers. Dextranase 3 was obtained from two Louisiana sugar factories on the day of consignment, as well as the supplier. Dextranase 4 was obtained from one Louisiana factory on the day of consignment.

Dextranase Activity

This was initially determined by three different methods. A spectrophotometric method (Anon, 2002a) measured reducing sugar from dextranase action on dextran (T2000TM, Amersham MW≥2,000,000 Da), which reacts with 3,5 dinitrosalicylic acid to give a yellow-brown color, which is measured at 540 nm. One dextranase unit (DU) is the amount of enzyme which degrades dextran to produce reducing sugar equivalent to 1 mg maltose per hour at 40°C and pH 5.4. A titration method (Anon, 2002b) was modified (see Appendix 1) to measure reducing sugar from

dextranase action on dextran T2000TM, and the reducing sugar is determined by the Hanes method. One dextranase unit (DU/mL) is the amount of enzyme which degrades dextran T2000TM to produce reducing sugar corresponding to the reducing power of one micromole of sodium thiosulfate in one min at 37°C and pH 5.8. Relative dextranase activities of the different enzymes studied were confirmed by comparison of ion chromatograms, after dextran (T2000TM; 500ppm) was digested by the dextranase (0.015g/100ml acetate pH 5.4 buffer [13.5g sodium acetate trihydrate was dissolved in 900ml of deionized water, and the pH adjusted to pH 5.4 with 1N acetic acid]) for 30min at 40°C in a shaking (90 rpm) water bath.

Haze Dextran in Sugarcane Syrups

Haze dextran in sugarcane syrups was determined following an alcohol method (ICUMSA GS1-15 [1994]) with modifications. Dextran T2000TM was the standard and dextran was precipitated with 100% absolute ethanol.

Haze Dextran in Sugarcane Syrups

Haze dextran in sugarcane juices was based on ICUMSA GS1-15 [1994] and rapid haze (Clarke et al, 1987) methods with modifications. Juice (35ml) was pipetted into a conical flask and TermamylTM (Novo, U.S.) amylase (0.1 ml) added at 55°C for 15 min to degrade starch. The mixture (10 ml) was then pipetted into a 25 ml plastic syringe with a filter holder attached, containing a coarse glass filter (25 mm). TCA (10%) solution (2 ml) was then pipetted into the syringe body and 0.5 g high-grade celite filter aid (Aldrich, U.S.). The plunger was placed in the syringe, and inverted at least five times. The first 2 ml of filtrate was discarded, and then 5 ml filtrate was added to 5 ml absolute ethanol, mixed and left for 2 min. The absorbance at 720 nm was immediately read in a 1 cm cell on a Shimadzu UV/VIS-1201 spectrophotometer. The amount of dextran was calculated using the same standard curve as for the ICUMSA haze dextran method above.

°Brix The mean °Brix of triplicate samples was measured using an Index Instruments TCR 15-30 temperature controlled refractometer accurate to ± 0.01°Brix.

Dextran Breakdown Products by Ion Chromatography with Integrated Pulsed Amperometric Detection (IC-IPAD)

See (Eggleston, 2002) for method. Duplicate samples were diluted (1g/25ml) then filtered through 0.45 µm filters. All compounds analyzed were quantitated in reference to standards.

Viscosity

The viscosity of syrup was measured on a Brookfield (Middleboro, U.S.) DV-II⁺ rotational viscometer at 25°C using spindle no. 18 (see Eggleston et al, 2004).

Temperature Effect Studies

Pure Dextran: Dextran T2000TM (500ppm) was prepared in pH 5.4 acetate buffer. Dextranase enzymes 2, 3 & 4 were first diluted (0.015g/100ml pH 5.4 acetate buffer). The "concentrated" dextranase 2 had also been previously diluted 4.6X to make it economically equivalent to the nearest priced "non-concentrated" dextranase. Diluted enzyme (1ml) was added to the dextran (2 ml) in a test-tube, covered with aluminum foil, and thoroughly mixed. For the control, deionized water (1 ml) was added instead of dilute dextranase. The test-tubes were placed in a shaking

waterbath (90 rpm) at different temperatures ranging from 26.6 - 65.5°C for 25min. After incubation the test-tubes were immediately placed in a boiling water-bath for 2.5 min to denature the enzyme, and cooled on ice. Preliminary experiments were undertaken to ensure that 2.5 min boiling was sufficient time to stop further dextranase action. Undiluted duplicate aliquots were analyzed on IC-IPAD.

Sugarcane Juice: Mixed juice (12.7°Brix; pH 6.1; 3177 ppm haze dextran/°Brix) was obtained from a Louisiana factory and stored in a -80°C freezer until used. Dextranases 2, 3 & 4 were first diluted (0.03g/100ml) in pH 5.4 acetate buffer. The "concentrated" enzyme 2 had also been previously diluted 4.6X to make it economically equivalent to the nearest priced "non-concentrated" dextranase. Diluted enzyme (1 ml) was added to the juice (2 ml) in a test-tube, covered with aluminum foil, and thoroughly mixed. For the control, de-ionized water (1 ml) was added instead of dilute enzyme. The test-tubes were placed in a shaking water bath (90 rpm) at different temperatures ranging from 26.6-65.5°C for 25 min. After incubation the test-tubes were immediately placed in a boiling water-bath for 2.5 min, and cooled on ice. Duplicate aliquots were diluted (0.75ml/50ml de-ionized water) and filtered before IC-IPAD analyses.

[°]Brix Effect Study

Solutions of dextran T2000TM (1000 ppm) were prepared in pH 5.4 acetate buffer with differing [°]Brix levels. Dextran was added to the buffer first and completely dissolved by boiling for 2min, then sucrose was added to adjust the [°]Brix, and the final [°]Brix noted. A range of [°]Brixes was achieved: 0.0-70.6. Dextranases 2, 3 & 4 were first diluted (0.03g/100ml) in pH 5.4 acetate buffer; the "concentrated" dextranase 2 had been previously diluted 4.6X to make it economically equivalent to the nearest priced "non-concentrated" dextranase. Diluted enzyme (1 ml) was added to the dextran/sucrose solution (2 ml) in a test-tube, covered with aluminum foil, and thoroughly mixed. For the control, de-ionized water (1 ml) was added instead of dilute enzyme. The test-tubes were placed in a shaking water bath (90r pm) at 48.0°C for 25 min. After incubation the test-tubes were immediately placed in a boiling water-bath for 2.5 min, and cooled on ice. Diluted aliquots (dilution depended on the [°]Brix level) were analyzed on IC-IPAD.

Laboratory Dextranase Studies on Factory Syrups and Juices

Dextranase/Syrup Reactions: A final evaporator syrup (FES) was obtained from a Louisiana factory ([°]Brix 58.8, pH 6.4; 7230 ppm haze dextran/Brix) at the end of the 2002 grinding season, and stored in a -80°C freezer until used. Levels of dextranase addition were calculated as ppm/solids (ppm/solids x 4.5 = ppm/juice). "Non-concentrated" dextranase 3 (0, 20, 40 and 80 ppm/solids) was added to 200 ml of syrup and vortex mixed for 40 sec to ensure thorough mixing. The syrup/dextranase mix was then immediately placed in a shaking water bath at 63°C and 90 rpm. Aliquots (40 ml) were removed after 0, 10, 15 and 20 min, and boiled immediately for 2.5 min. After cooling, the samples were analyzed, in duplicate, for haze dextran and viscosity. Preliminary results showed that boiling did not affect the haze dextran or viscosity. For the "concentrated" dextranase 2, the reaction conditions were the same, except 10 ppm/solids was also analyzed.

Dextranase/Juice Reactions: A sugarcane juice (15.0°Brix; pH 5.6) was obtained for this study by allowing a sugarcane pile to deteriorate at ambient conditions for 3 days outside a Louisiana factory. Juice was extracted in the factory laboratory core press, and biocide added (20 ppm/juice; Bussan881TM, Buckman Labs.) to prevent further dextran formation reactions. A required level of

haze dextran (3380 ppm/ $^{\circ}$ Brix) was achieved by diluting this deteriorated juice with fresh crusher juice. "Non-concentrated" dextranase 3 (0, 10, 20, 40 and 80 ppm/juice) was added to 250ml of juice and mixed thoroughly for 40sec. The juice/dextranase mix was then immediately placed in a shaking water-bath at 32.2 $^{\circ}$ C and 90 rpm. Aliquots (40 ml) were removed after 0, 5, 10, 15 and 20 min, and boiled immediately for 2.5 min. After cooling, the samples were analyzed, in duplicate, for haze dextran. Preliminary results showed that boiling did not affect the haze dextran. This experiment was repeated at 48.9 $^{\circ}$ C. For the "concentrated" dextranase 2, the reaction conditions were the same.

Effect of Biocide on Reactivity of Dextranase

The method described above for dextranase/juice reactions was used to measure the effect of adding 0, 10 and 20 ppm biocide (dithiocarbamate; Bussan881TM) to juice (3380 ppm/ $^{\circ}$ Brix) in the presence of 10 ppm/juice "concentrated" dextranase 2.

Storage Characteristics of Dextranases

Fresh "non-concentrated" and "concentrated" dextranases (150ml) were stored in dark brown bottles in a cool and dark laboratory corner (ambient temperatures ranged from 23-27 $^{\circ}$ C). The activity of the dextranases was measured periodically over a 90 day period using the simple titration method (Appendix 1). As a control, samples of the same dextranases were stored in a refrigerator (4 $^{\circ}$ C) and periodically analyzed as well.

RESULTS AND DISCUSSION

Dextranases Available in the U.S.

Most commercial dextranases currently available in the U.S. are produced from a fungal source, *Chaetomium gracile* or *erraticum*, and have GRAS (Generally Recognized As Safe) status. The U.S. Food and Drug Administration does not approve of dextranases from *Penicillium* or bacterial sources which are sold overseas. Dextranases from *Chaetomium* have been shown to hydrolyze faster than dextranases from *Penicillium* and *Bacillus* sources (Taylor et al, 1990). One of the greatest sources of confusion for factory staff about dextranase is that it is not possible to directly compare the activities (or strengths) of commercial dextranases because each dextranase producer/vendor/distributor uses a different method to measure activity. For example, units of dextranase activity can be u/g, Du/g, U/ml and Du/ml. This is further exacerbated by the fact that the dextranase market is very dynamic and dextranase activities and prices can change regularly.

Consequently, there is an urgent need for a uniform method to measure dextranase activities at the factory. For this reason, a simple titration method to determine the relative activity of dextranase was identified (Anon, 2002b) and modified for easy use at the factory (see Appendix 1). The method does not need any sophisticated equipment, such as a spectrophotometer, and there is no need for standards and a standard curve. Having an easy-to-use method to measure activity will allow factory staff to compare commercial dextranases and make more informed decisions on which dextranase to use. The stability of the dextranase stored across the grinding season can also be monitored.

To undertake a study of factory optimization of dextranase application, it was first necessary to

understand the differences in the activities and properties of the different commercial dextranases available in the U.S. Relative activities and some physico-chemical properties of commercial dextranases that are currently available in the U.S. are listed in Table 1. Initially, a spectrophotometric (Anon, 2002a) and the titration method were used to compare the activities of commercial dextranases (Table 1), and the correlation between the two was excellent ($R^2=0.999$) which confirms their accuracy. It was also found that the relative activities of commercial dextranases can be accurately confirmed using IC-IPAD profiles of dextran/dextranase mixtures, and these are illustrated in Figure 1. Dextranase breaks high MW dextran down into smaller, more manageable MW dextran and eventually oligosaccharides, with isomaltotriose, isomaltose and glucose being the final major products (Figure 1).

Table 1. Relative Activities of Dextranases and Some Physico-Chemical Properties

Commercial Dextranase	Source	Rel. Specific Activity DU/g ^a	Rel. Specific Activity DU/ml ^b	Color	Brix	Protein % w/w
“Concentrated” 1	<i>Chaetomium gracile</i>	60,603.3	48,072.1	Clear, light tan	45.13	0.84
“Concentrated” 2	<i>Chaetomium erraticum</i>	n.d. ^c	57,686.5	Clear, light tan	41.96	n.d.
“Non-concentrated” 3 ^d (Factory 1)	<i>Chaetomium gracile</i>	8,402.8	5,999.4	Clear, slightly yellow	26.57	0.26
“Non-concentrated” 3 ^d (Factory 2)	<i>Chaetomium gracile</i>	8,290.8	5,499.5	Clear, slightly yellow	26.64	0.15
“Non-concentrated” 4	<i>Chaetomium gracile</i>	6,356.3	4,783.2	Clear, v. slightly yellow	37.45	0.12

^a See Anon (2002a)

^b See Anon (2002b) and Appendix 1

^c n.d. = not determined

^d “Non-concentrated” dextranase 3 was obtained from barrels which had been delivered to Factory 1 and 2 that day, and stored at 4°C immediately. A sample of this enzyme was also sent from the supplier and a similar 5,499.5 DU/ml obtained.

The greater the dextranase activity, the greater the number and size of the breakdown product peaks that occur on the ion chromatograms. Considerable variation existed among the enzymes currently being used in Louisiana and others available in the U.S. (Table 1). Dextranases could be categorized into “concentrated” and “non-concentrated” forms, and an approximate 8-10 fold difference in activity existed between the two forms, but this did not always reflect the corresponding differences in price and highlights the different practices of distributors. Activity variations also existed among dextranases within each concentration form. As expected, the

“concentrated” dextranases produced more and larger IC peaks than the “non-concentrated” dextranases currently being used in Louisiana (Figure 1). Because of the ease and simplicity of the titration method compared to the spectrophotometric method, subsequent enzyme activities in this research were only measured using the titration method.

Current Addition of Commercial Dextranases in the U.S. - Survey Results

To gain information on the current status of dextranase applications in Louisiana a questionnaire/survey was sent out to all Louisiana factories in 2002. Eleven out of sixteen factories responded and results are summarized in Table 2. All applications varied considerably with factory. Some factories chose not to use dextranase, but rather opted to manage dextran by managing cane cut-to-crush times. Only “non-concentrated” dextranases were being used in Louisiana factories in 2002 and 2003. Most dextranase applications were in the last evaporator bodies at very low ppm levels (2-10 ppm/syrup), with one factory adding it to massecuites in a vacuum pan (Table 2), and the others to juice. Only one factory had an incubation tank ($R_t \sim 12$ min) for dextranase addition, although it was installed to aid the natural amylase activity of sugarcane (Eggleston et al, 2003).

Factors Which Affect Dextranase Activities in the Factory

The optimum pH range for dextranase activity is pH 5.0-6.0, with the lower end of the range more preferable, and this coincides with the typical pH operating range of juices before clarification in U.S. sugarcane factories. Therefore, there was no need to manipulate juice pH. Addition of lime in the clarification process increases the pH well above pH 6.0, indicating that dextranase should never be added to limed juice. Syrup pH is often between pH 6.0-6.5, which is less optimal than natural juice pH. For these reasons, pH effects were not studied; instead we focussed on the effects of temperature and °Brix.

Dextranase Activity at Different Temperatures

Temperature is known to have a critical effect on the activity of dextranases (Inkerman, 1980). We undertook studies on the effect of temperature on the activity of dextranases using IC-IPAD with relative dextranase activity being measured as the isomaltotriose peak height. The pattern of temperature/dextranase effects was close for pure dextran (Fig. 2a) and dextran formed in juice (Fig. 2b), indicating T2000 was an adequate standard. The “concentrated” enzyme was added after a 4.6 dilution to make it economically equivalent to the nearest “non-concentrated” dextranase. However, even at this dilution the activity was still consistently higher across the temperature range studied than for the “non-concentrated” dextranases (Fig. 2), which highlights the differential between relative economic price and activity. The remarkable similarity of the temperature effects patterns for the “concentrated” and “non-concentrated” dextranases studied (Fig. 2), most likely highlight the same fungal source of the dextranases (Table 1).

For all the dextranases studied the maximum activity was distinctly ~48.9°C, with 43.3-54.4°C being the optimum activity range. It has been reported by Morel du Boil and Wienese (2002) that high °Brix levels may increase the stability of dextranase to slightly higher temperatures. The lowest activity occurred at ~65.5°C, because of partial denaturation of the dextranase enzyme. Dextranase activities were also low at 26.7-32.2°C, but were still better than at ~65.5°C. This has dramatic consequences for the factory application of dextranases. Most last evaporators, where many factories have been adding dextranase (Table 2) have syrup temperatures ~65.5°C, but the activity has dropped off dramatically at this temperature (Figure 2). Even for those factories that

activity has dropped off dramatically at this temperature (Figure 2). Even for those factories that are adding dextranase to juice (Table 2) where the ambient juice temperatures are ~26.7-32.2°C, the dextranase activity is still relatively low (Figure 2).

Table 2. Results of dextranase factory application survey conducted in 2002

Factory ^a	Dextranase used	Level added (ppm/juice or syrup) ^a	Point of Factory Addition in 2002	Temp. °C	Resid. Time (min)	pH
1.	“Non-Concentrated” 4	5-10	Last evaporator body	62.8	20	6.5
2.	“Non-Concentrated” 3	8	Low grade vacuum pan	65.5	not known	5.9
3.	“Non-Concentrated” 5	5-20	Juice incubator tank	65.5	12-14	5.0-5.4
4.	“Non-Concentrated” 3	10	3rd evaporator body	60	30	6.0
5.	“Non-Concentrated” 3	2-4 ppm/ton juice	Juice tank under cush-cush	29.4	10	5.4-5.6
6.	“Non-Concentrated” 3	2-4 ppm/ton	Between 3-4th evaporators	76.7-79.4	not known	6.4
7.	“Non-Concentrated” 5	5	3rd effect evaporator	60-65.5	not known	6.5
8.	“Non-Concentrated” enzyme 5	2	Syrup body	60	10-30	6.1-6.2
9.	NONE	--	--	--	--	--

^a Two other factories also responded that they did not add dextranase

Effect of °Brix on Dextranase Activity

Similar to the temperature effects (Figure 2), even though the “concentrated” enzyme was pre-diluted to the economic equivalence of the nearest priced “non-concentrated” dextranase, the activity was still much higher at different °Brixes, which further highlights the differential between economic price and activity. The activity of the dextranases was stable up to ~25-30°Brix, but afterwards decreased dramatically. This is because as the °Brix increases there is a depletion of water, i.e., the water activity decreases, which is the second substrate in the hydrolysis of dextran by dextranase. Another contributing factor could be the concentration of impurities which inhibit dextranases. As can be seen in Figure 3, the dextranase activity at °Brixes of evaporator syrups was very low. This, compounded with the high temperatures that are known to inhibit dextranase (Figure 2), strongly indicates that the last evaporator is not the best point to add dextranase in the U.S. Most certainly, dextranase cannot be added in massecuites where the °Brix is often ~80. In contrast, juices before clarification occur from ~12-15°Brix and would, therefore, not suffer such °Brix inhibitory affects on dextranases.

Dextranase Studies on Factory Syrups and Juices

Application of Dextranases to Last Evaporator Effect Syrup

Because of the lack of adequate residence times for the addition of juices in U.S. factories, dextranase has been added to the last evaporators, mostly because of the availability of up to 20 min calculated R_t (Eggleston and Monge, 2002). In raw sugar factories, solids are concentrated from approximately 15.0°Brix in juice to 65.0°Brix in final evaporator syrup. As a consequence, the dextranase has to act upon ~4.5 times as much dextran in syrup than in juice.

For this reason, we added dextranase to syrups as ppm on solids (i.e., equivalent to 4.5 times as much as if it had been added on juice). Dextran removal was measured using a modified haze dextran method because of its ease of use and because it gives a reliable estimate of high MW dextrans which are responsible for the major processing difficulties associated with this polysaccharide (Inkerman, 1980). However, the haze method is a reflection of all haze forming material, and although proteins are precipitated with trichloroacetic acid and starch is removed with amylase, other polysaccharides such as indigenous cane polysaccharide could have contributed to the haze.

Therefore, although results are relative, they are most likely underestimations of dextran removal. Viscosity was monitored as well as % dextran removal, to ensure that processing efficiency was definitely improved upon by degrading dextran in syrup with dextranase. The effect of various concentrations of a “non-concentrated” dextranase to a final evaporator syrup containing dextran (7230 ppm/°Brix) at 63°C are shown in Figure 4. It can be seen in Figure 4a that 20 ppm/solids dextranase had very little effect on dextran degradation, with only 7.4% being removed after 20 min. Slight improvements occurred at 40 ppm (Figure 4a) with 15.8% dextran removal after 20 min. Dextran degradation with 80 ppm/solids dextranase was better, with 13.5 and 25.2% dextran removal after 15 and 20 min, respectively. However, “non-concentrated” dextranase at 80 ppm/solids is equivalent to 360 ppm/juice, and for such a limited breakdown in dextran at this high level, it is not economically viable to add “non-concentrated” dextranase to syrup. The effect of the “non-concentrated” dextranase on viscosity reduction in the syrup is illustrated in Figure 4b. At 0 ppm dextranase, the viscosity increased slightly across 20 min R_t , most likely because of

evaporation. No significant affect on viscosity was found at 20 ppm/solids. It was only at 40 and 80 ppm/solids addition of the “non-concentrated” dextranase, that viscosity reductions were found (Figure 4b). Similar small viscosity reductions of syrup in the presence of dextranase were found by Hidi and Staker (1975), as the viscosity of syrups is mostly caused by sucrose. These results agree with initial factory studies that we conducted where no differences were observed in viscosity reductions by adding 20 ppm/solids of another “non-concentrated” dextranase to a last evaporator body (65°C ; $R_t = 20 \text{ min}$) over the control of 0 ppm/solids.

Compared to the “non-concentrated” dextranase (Figure 4), a much lower level (10 ppm/solids) of the “concentrated” dextranase was able to remove 37% of dextran after 20 min R_t (Figure 5a). As expected, the higher the level of “concentrated” dextranase applied, the more dextran removal occurred (Figure 5a), but at levels >10 ppm/solids the economics of the applications are not favorable. Furthermore, even at 10 ppm/solids, after 15 min R_t , a significant reduction in viscosity was observed (Figure 5b). 10 ppm/solids is equivalent to 45 ppm/juice, which is much higher than the levels at which factories have been adding “non-concentrated” dextranases in Louisiana (Table 2).

Application of Dextranases to Juice

Current factory applications of dextranase to juice occur at ambient temperatures $\sim 32.2^{\circ}\text{C}$ (90°F), but the maximum activity of dextranases in juice was $\sim 48.9^{\circ}\text{C}$ (120°F ; Figure 2). Consequently, we investigated the application of both the “non-concentrated” and “concentrated” dextranases that were previously studied on syrup, to juice (3380 ppm dextran/ $^{\circ}\text{Brix}$) at both 32.2 and 48.9°C . R_t s up to 25 min were also studied because one Louisiana factory had expressed interest in building a large incubation tank with a such a R_t . However, most applications of “non-concentrated” dextranases to juice occur in Louisiana with available R_t of only ~ 10 min or less (Table 3).

Current factory applications of “non-concentrated” dextranases to juices at 32.2°C have mostly occurred at 2-4 ppm/juice levels (Table 2). However, as can be seen in Figure 6a, at 4 ppm/juice and after 10 min R_t , only 11.8% dextran had been removed. Increasing the dextranase level to 10 ppm/juice slightly improved dextran removal (Figure 6a), and even at 20 ppm/juice only 26.7% dextran was removed after 10 min. Markedly more dextran was removed from the juice by the “non-concentrated” dextranase when the reaction occurred at 48.9°C (Figure 6b). After 5 min at 4 ppm/juice and 48.9°C , $\sim 19.6\%$ dextran was removed compared to only 4.7% at 32.2°C (Figure 6a). After 5 min at 10 ppm/juice and 48.9°C , $\sim 23.1\%$ dextran was removed compared to 11.4% at 32.2°C , and so on (Figure 6). Results, therefore, indicate that heating the juice to the maximum dextranase activity temperature of 48.9°C , markedly improves the efficiency of application and, to some extent, overcomes the limited availability of R_t in many factories.

In comparison to the “non-concentrated” dextranase at 32.2°C (Figure 6a), the “concentrated” dextranase performed markedly better at this ambient juice temperature (Figure 7a). At just 4 ppm/juice and after 5 min R_t at 32.2°C , a remarkable 29.6% dextran was removed by the “concentrated” dextranase (Figure 7a) compared to only 4.7% for the “non-concentrated” dextranase under the same conditions (Figure 6a). Increasing the “concentrated” dextranase level to 8 ppm/juice markedly increased dextran removal to $\sim 50\%$ at 32.2°C and 5 min R_t (Fig. 7a). Little

further effect was obtained at >8 ppm/juice of the “concentrated” dextranase (Figure 7a), with most of the dextran had been broken down after 5 min by 10 ppm/juice.

Heating the juice and “concentrated” dextranase mixture to 48.9°C also caused a dramatic improvement (Figure 7b), and for this reason we could study the effects over a shortened R_t of 15 min. Even after 5 min at 4 ppm/juice and 48.9°C, most of the dextran measured by the haze method was removed (Figure 7b). “Concentrated” dextranase >4 ppm/juice at 48.9°C had very little further effect (Figure 7b) as most of the dextran substrate had been depleted and, therefore, 4 ppm/juice was an overdose because of asymptotic behavior under the stated conditions. Consequently, levels as low as 2 ppm/juice of the “concentrated” dextranase after only 5 min will be sufficient in juice at 48.9°C. Before addition at such a low levels, the “concentrated” dextranase should be first diluted with stabilizing sucrose solution to increase enzyme-substrate contact time. These results, therefore, strongly suggest that heating the juice to the max. dextranase activity temperature of 48.9°C, markedly improves the efficiency and economics of application and, to some extent, overcomes the limited availability of R_t in many factories.

Addition of Dextranases to Juice in the Presence of Biocide

One question that arises about the application of dextranases at the higher temperature of 48.9°C is will such a temperature stimulate adverse microbial growth and possible dextran formation? It may be necessary to simultaneously add biocide and dextranase to the juice. For this reason we investigated the effect of dextranase in the presence of a biocide (dithiocarbamate) commonly used in U.S. factories at approximately 10 ppm/juice levels. Results shown in Figure 8 show that the “concentrated” dextranase still worked in the presence of the biocide, and 10 ppm levels of biocide may be slightly more favorable than 20 ppm additions. When severe dextran problems occur, an option in the factory could be to add 10 ppm/juice to the mill tandem and 10 ppm to mixed juice.

Storage Characteristics of “Non-Concentrated” and “Concentrated” Dextranases Under Simulated Factory Conditions

Dextranase vendors/distributors routinely recommend that barrels of dextranase should be stored in the coolest and shadiest area of the factory in order to prevent loss of activity at higher temperatures. To simulate such factory storage conditions we stored a “non-concentrated” and “concentrated”dextranase in a cool and dark corner of a laboratory (ambient temperatures range from 23-27°C), and analyzed the effect of storage time on the dextranase activity across the approximate length of a grinding season (~90 days). By the end of ~90 days, the activity of the “concentrated” dextranase had decreased only slightly (~9%; Figure 9). In dramatic contrast, the activity of the “non-concentrated” dextranase decreased dramatically across the storage time under these simulated factory conditions (Figure 9), and the activity was approximately half (~46%) of what it was at the beginning. This means that the level of application of the “non-concentrated” dextranase in the juice would have to be doubled in the late season if the same break down of dextran was to be achieved. This is of concern to Louisiana factories in particular, as freezes often occur that can cause severe dextran problems. The dramatic decrease in activity for the “non-concentrated” dextranase is most likely due to excessive dilution water de-activating/denaturing the enzyme protein structure, or by increasing conformational mobility of the protein.

As a control, the dextranases were also stored in a refrigerator (4°C; Figure 10). Whereas there was no change in activity for the “concentrated” dextranase stored under refrigeration, for the “non-concentrated” dextranase the activity still decreased significantly (Figure 10) but to a lesser extent than the simulated factory conditions conditions (Figure 9). There are no ideal or easy storage conditions for “non-concentrated” dextranase at the factory, and factories may want to consider not purchasing all their dextranase early in the season. Furthermore, this problem of the storage of “non-concentrated” dextranase highlights the need for the factory staff to be able to monitor the activity of their dextranase across the season.

Economic Costs of Different Dextranase Applications

The relative equivalent cost for the “concentrated” versus the “non-concentrated” was greatest in the syrup (Table 3), with \$2.55 spent on the “non-concentrated” dextranase for every US dollar spent on the “concentrated” one. Irrespective of the dextranase concentration form used, much higher levels of dextranase had to be added to syrup compared to the juice at either 32.2°C or 48.9°C (Table 3). Cost calculations of dextranase juice addition after 5 min R_t were undertaken because of the limited amount of R_t currently available in U.S. factories. At 32.2°C, a 4 ppm/juice level of the “concentrated” dextranase was approximately two-fold less expensive compared to the “non-concentrated” dextranase (Table 3). Although the difference in cost-effectiveness between the two concentration forms of dextranase apparently became much smaller at 48.9°C and 4 ppm/juice levels (Table 3), this is misleading. This is because at 4 ppm/juice the “concentrated” dextranase was overdosed (Figure 7b), with very little extra dextran substrate being removed at higher ppm levels or R_t s. The “concentrated” dextranase can, therefore, be added at even lower levels, i.e. <4 ppm/juice, compared to the “non-concentrated” dextranase (Figure 6), which is more economical. Furthermore, these cost calculations of application do not take into account the extra freight cost of the “non-concentrated” dextranase which contains more water.

SUMMARY AND FURTHER DISCUSSION

In the U.S., most commercial dextranase is from a fungal (*Chaetomium*) source and is available in “non-concentrated” or “concentrated” forms. An approximate 8-10 fold difference in activity exists between the two forms, and activity variations exist among dextranases within each form. Currently, there is no uniform method to measure dextranase activity used by commercial dextranase producers/vendors/distributors, which has meant that direct comparison of activities is not possible. A simple titration method to determine the relative activity of dextranases has been identified and modified for easy factory use. This method will allow factory staff to compare commercial dextranases and make more informed decisions on which dextranase to use, as well as monitor the stability of the dextranases stored across the grinding season, as some have been shown to change. Under factory storage conditions over the typical length of a Louisiana grinding season, the activity of a “concentrated” dextranase decreased only slightly (~9%). In strong contrast, the activity of a “non-concentrated” dextranase had approximately halved (~46%) and even reduced in activity when stored under refrigeration.

Table 3. Cost-effective calculations for different dextranase applications.

**“Concentrated” dextranase based on \$18.36 per lb
“Non-concentrated” dextranase based on \$6 per lb**

**Syrup (7230ppm/Brix) Application
63°C and 20 min R_t**

Dextranase	ppm/solids ^a	Dextran Breakdown	Breakdown Ratio	Relative Equivalent Cost
“Concentrated”	20	57.3%	1	\$1
“Non-concentrated”	20	7.4%	7.79	\$2.55

^a 20 ppm/solids is equivalent to 90ppm/juice

**Juice (3380ppm/Brix) Application
32.2°C and 5 min R_t**

Dextranase	ppm/juice	Dextran Breakdown	Breakdown Ratio	Relative Equivalent Cost
“Concentrated”	4	29.6%	1	\$1
“Non-concentrated”	4	4.7%	6.30	\$2.06

**Juice (3380ppm/Brix) Application
48.9°C and 5min R_t**

Dextranase	ppm/juice	Dextran Breakdown	Breakdown Ratio	Relative Equivalent Cost
“Concentrated”	4 ^a	61.2%	1	\$1 ^a
“Non-concentrated”	4	19.6%	3.12	\$1.02

^a The “concentrated” dextranase was overdosed at 4 ppm/juice because of an asymptotic response - see text for full details.

Overall, applications of dextranases to juice were much more efficient and economical than adding them to evaporator syrups, and application of “concentrated” dextranase was more economical than application of “non-concentrated”. Some sugar technologists (DeStefano, 1988) have advocated dextranase addition to syrup rather than juice because it is “wasteful to treat contaminated material before its clarification”. However, recent large factory studies (Eggleston et al, 2003) have shown

that dextran removal is very dependent on the clarification system in use, and if no pre-heating of the juice occurs, as in cold liming, dextran can even form. Furthermore, Eggleston et al (2003) found much less dextran removal across factory clarification processes than DeStefano (1988) reported in three samples. Application of “non-concentrated” dextranases to final evaporator syrups is not economical and confirms initial factory studies. However, “concentrated” dextranase can be applied at levels as low as 10 ppm/solids (equivalent to 45 ppm/juice), and although this is higher than levels required for addition to juice, factory staff could consider adding it to both syrup and juice when severe dextran problems occur, such as after a severe freeze or storm. Heating the juice to 48.9°C, dramatically removed more dextran from a juice than at the current ambient temperature of application (32.2°C) and was more economical.

In practice at the factory, to bring the juice temperature up to 48.9°C (or at least in the optimum temperature range of 43.3-54.4°C), heated juice could be recirculated into small tanks with as little as 5 min Rt for the “concentrated” dextranase to work at <4 ppm/juice levels. Such tanks could include tanks which were previously used for cold liming. Accurate temperature control of the juice must be maintained to prevent inactivation of the dextranase. *Leuconostoc* and *lactobacillus* growth does not occur readily at or above a temperature of 50°C (Inkerman, 1980), therefore, the possible problem of simultaneous dextran formation and breakdown is expected to be limited. This is further evidenced by the formation of dextran being catalyzed by an exogenous enzyme dextranucrase, compared to the endogenous mechanism of dextranase to breakdown dextran. Moreover, dextranase was shown to work in the presence of biocide (dithiocarbamate) in juice. Nevertheless, factory studies are being planned to check that no adverse dextran formation is occurring at 48.9°C, as well as optimize conditions for factory conditions as extrapolation from the laboratory to the factory is not always simple.

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APPENDIX 1

Easy Measurement of Dextranase Activity at the Sugarcane or Sugarbeet Factory/Refinery

Modified (Anon, 2002b) Thiosulfate Titraton Assay

Principle:

Dextranase hydrolyzes the α 1,6 glucosidic linkages in dextran. Dextranase activity is assayed by letting it hydrolyze dextran and measuring the amount of reducing sugar formed. The reducing sugar is determined by the Hanes method. Equations for the reactions are as follows:

	<u>Reaction End Color</u>
1. $K_3Fe(CN)_6 + \text{Reducing Sugar} \rightarrow Na_2CO_3 \rightarrow K_4Fe(CN)_6$	yellow
2. $2K_3Fe(CN)_6 + 2KI \rightarrow \text{Acetic acid} \rightarrow 2K_4Fe(CN)_6 + I_2$	orange
3. $2K_4Fe(CN)_6 + 3ZnSO_4 \rightarrow K_2Zn[Fe(CN)_6]_2 \cdot + 3K_2SO_4$	orange
4. $I_2 + \text{starch indicator} \rightarrow \text{starch-I}_2 \text{ complex}$	dark blue
5. $\text{Starch-I}_2 \text{ complex} + 2NaS_2O_3 \rightarrow Na_2S_4O_6 + 2NaI$	white

Reaction conditions: Temperature - 37°C, pH - 5.8, Reaction time - 30 minutes

Definition of Units

1 unit of dextranase activity (DU) is defined as the amount of enzyme which degrades dextran to produce reducing sugar corresponding to the reducing power of one μM of sodium thiosulfate in one min at the conditions stated above.

Simple Equipment Required:

Water bath or constant temperature oven at 37°C
Metal pan for a boiling water bath
Burette (25 ml)

Boiling test-tubes
Stoppered, conical flasks (50ml)
Volumetric flasks

Reagents:

Dextran Solution

Weigh out the dry weight equivalent of 0.5 g Dextran T2000™ (Amersham) into a 100 ml volumetric flask. Make to mark with distilled or deionized water and dissolve. Boil if necessary to dissolve.

Reagent A

Separately, weigh out 8.25 g of potassium ferricyanide and 10.6 g of anhydrous sodium carbonate into weigh boats. Rinse both compounds into a 1 L volumetric flask with distilled or deionized water. Make up to the mark with distilled or deionized water. Pour the solution into a dark brown bottle and allow to stand in the dark for 2 or 3 days.

Reagent B

Separately, weigh out 25 g potassium iodide, 50 g zinc sulfate heptahydrate, and 250 g sodium chloride. Rinse all compounds into a 1L volumetric flask with distilled or deionized water. Make up to the mark with distilled or deionized water and allow the compounds to dissolve.

Reagent C

Put 50 ml of glacial acetic acid into a 1 L volumetric flask. Make up to the mark with distilled or deionized water.

1% Starch Indicator

Weigh out 1 g of soluble potato starch (Sigma) into a 100 ml volumetric flask. Rinse in with distilled water. Make up to about the 80 ml mark. Boil to dissolve then cool. Make up to the mark with distilled or deionized water.

0.1M Acetate Buffer, pH 5.8

Weigh out 13.5 g sodium acetate trihydrate into a boat. Wash this into a 1 L beaker with 900 ml millipore water. Adjust the pH to 5.8 with 1N acetic acid (approx. 11 ml). Transfer to a 1 L volumetric flask. Label and store at room temp. Replace after 4 weeks.

0.01N Sodium Thiosulfate

Accurately weigh out 1.241 g sodium thiosulfate pentahydrate into a boat. Rinse with distilled or deionized water into a 1 L volumetric flask. Make up to mark with distilled or deionized water.

Dilution of Dextranase Enzyme

The more active the enzyme is, the more dilution is required. Conversely, the less active an enzyme is, the less dilution is required. It is recommended that factories/refineries start at a dilution of 0.5g/L in de-ionized water:

Weigh out 0.5 g enzyme into a weigh boat. Rinse this into a 1 L volumetric flask with distilled water. Make up to the mark with distilled water.

For "concentrated" dextranases, a dilution of 0.065g/L de-ionized water is recommended.

Procedure

A. Pipette 10 ml of dextran solution into three test-tubes then pipette in 4 ml of 0.1M Acetate buffer, pH 5.8. Cover the test-tube in aluminum foil to prevent evaporation. Shake well. Label one test-tube as replicate (1), one test-tube as replicate (2), and the final test-tube as the control.

B. Add 1 ml of distilled water to the control test-tube. Add 1 ml of the dilute enzyme solution to test-tube (1) and (2). Shake well. Put in a water bath at constant temperature of 37°C and allow to incubate for 30 min.

C. Label three stoppered conical flasks the same as the three test-tubes.

D. Into each of the three stoppered conical flasks, pipette 5 ml of Reagent A and 3 ml of distilled or deionized water.

E. After 30 min of incubation, remove the test-tubes from the incubator/water bath. Pipette 2 ml of the incubated test-tube solution into the correct stoppered conical flasks and solutions. Close the conical flasks with their stoppers, shake, then place them in a boiling water bath for 15 min. Allow to cool.

F. To the stoppered conical flasks pipette in 5 ml of **Reagent B** first, then add 3 ml of **Reagent C**. Add 5 drops of the soluble starch solution. The solutions will turn dark blue after mixing. **This is solution X.**

G. 0.01N sodium thiosulfate has already been put into a burette. Titrate sodium thiosulfate into solution X until the blue color of the starch-iodine complex completely disappears. Make sure to swirl. Accurately write down the ml of sodium thiosulfate that were needed for the titration. Take the average ml of replicates (1) and (2).

Calculation of Dextranase Activity:

$$\text{Units/ml} = (C - T) \times 0.01 \times 1000 \times 1/30 \times 15/2 \times N$$

C= Titration ml of the control

T= Titration ml of the enzyme

N = Dilution multiple of enzyme

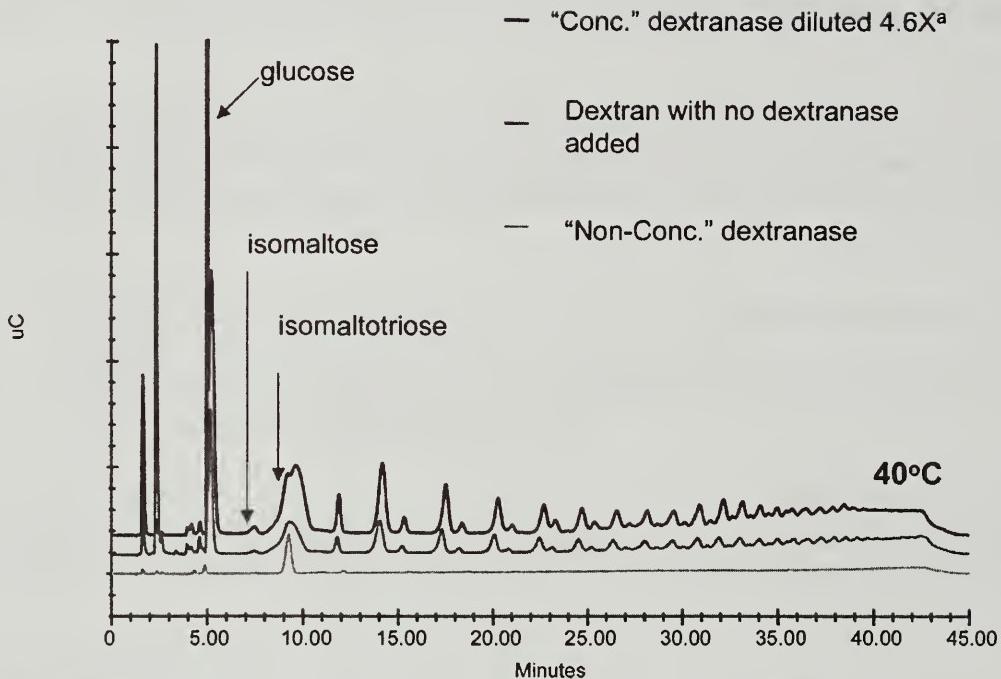


Fig. 1. Use of ion chromatography profiles to accurately determine relative dextranase activities

^a At the time of the experiment the "conc" dextranase cost 4.6X more than the "non-conc" dextranase. Since the experiment the difference is only 3.1X.

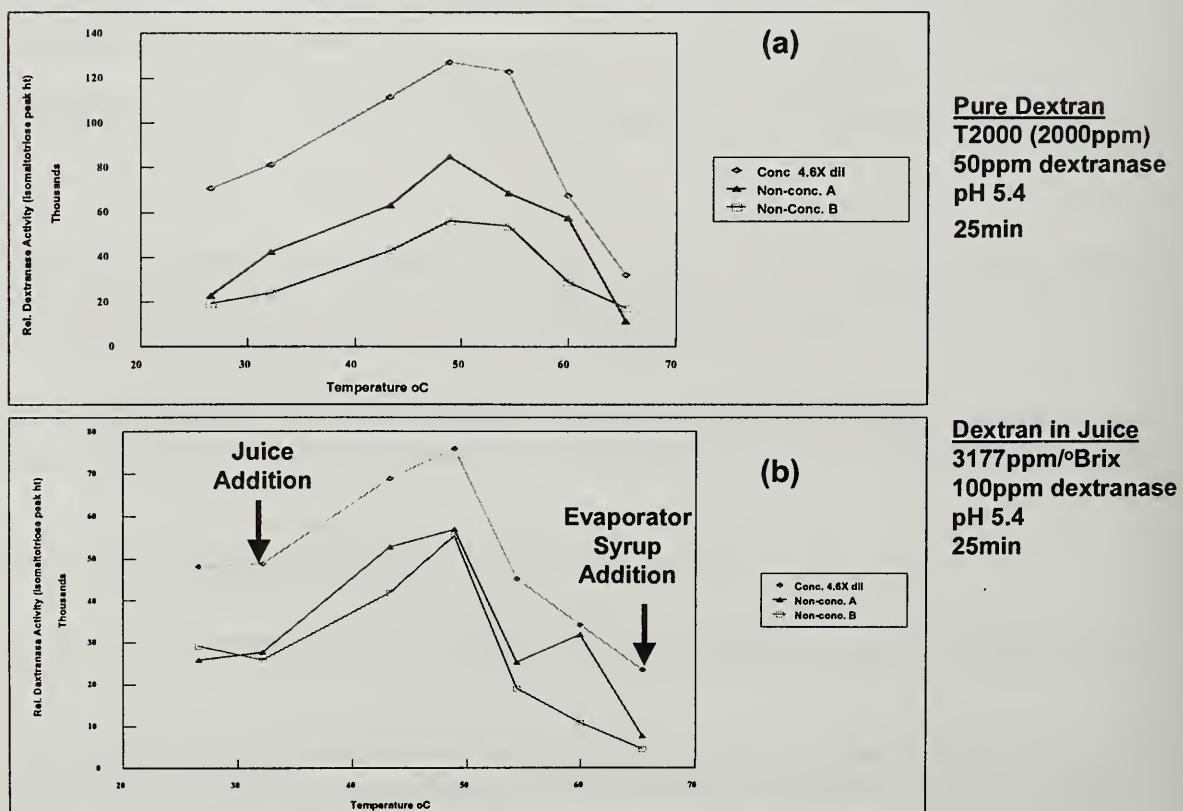


Fig. 2. Effect of temperature on dextranase activity to breakdown (a) dextran T2000™ and (b) cane juice dextran

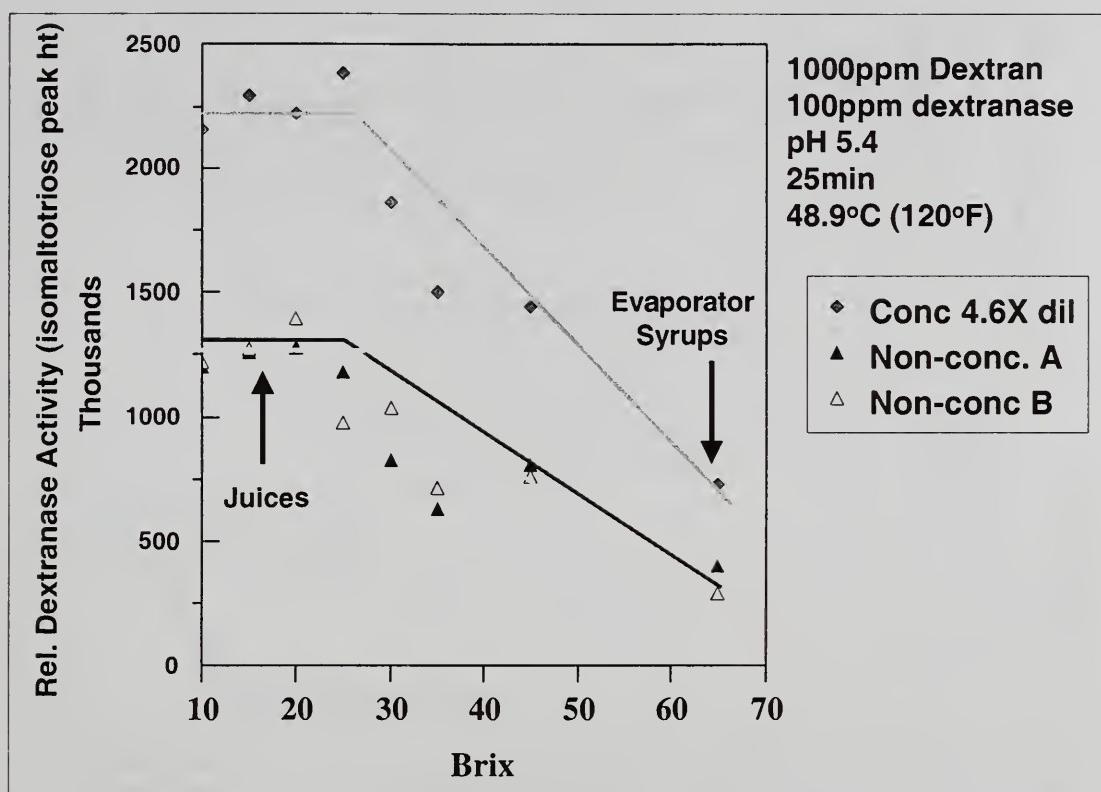


Fig. 3. Effect of °Brix on dextranase activity

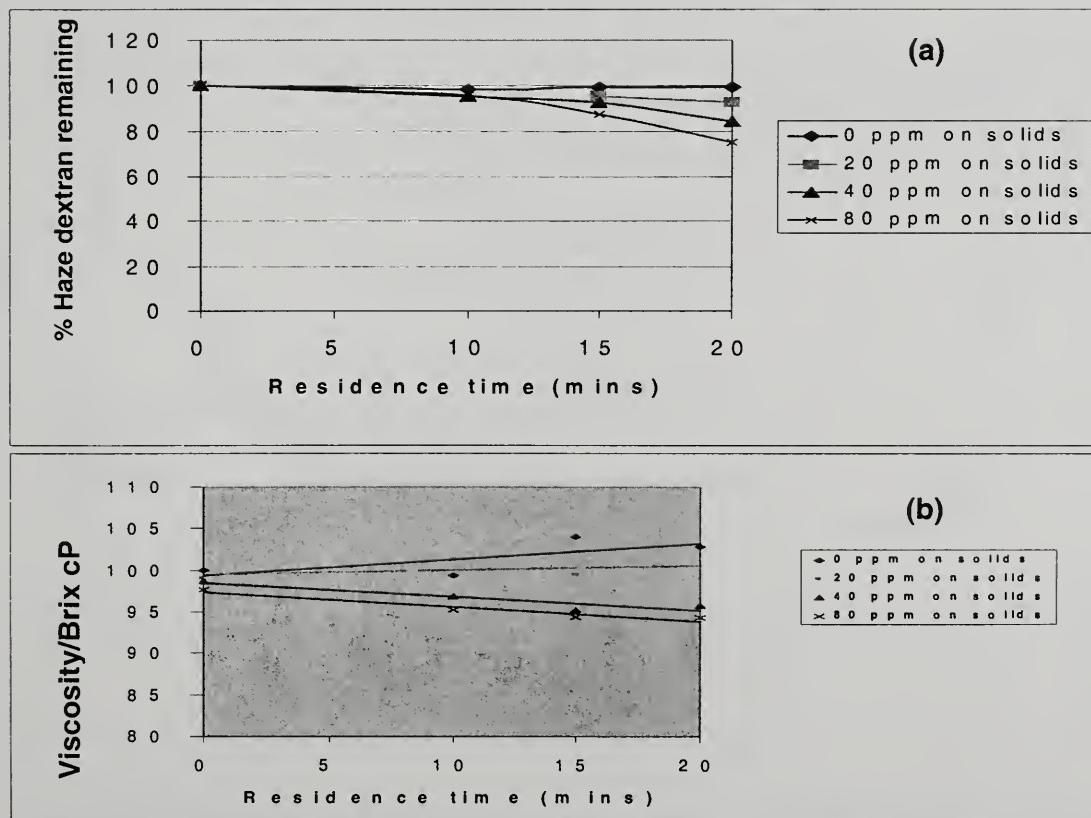


Fig. 4. Effect of various levels of "non-concentrated" dextranase in a final evaporator syrup (7230ppm/°Brix Dextran) on (a) the removal of dextran and (b) reduction in viscosity. (Note: ppm/solids is equivalent to 4.5 x ppm/juice).

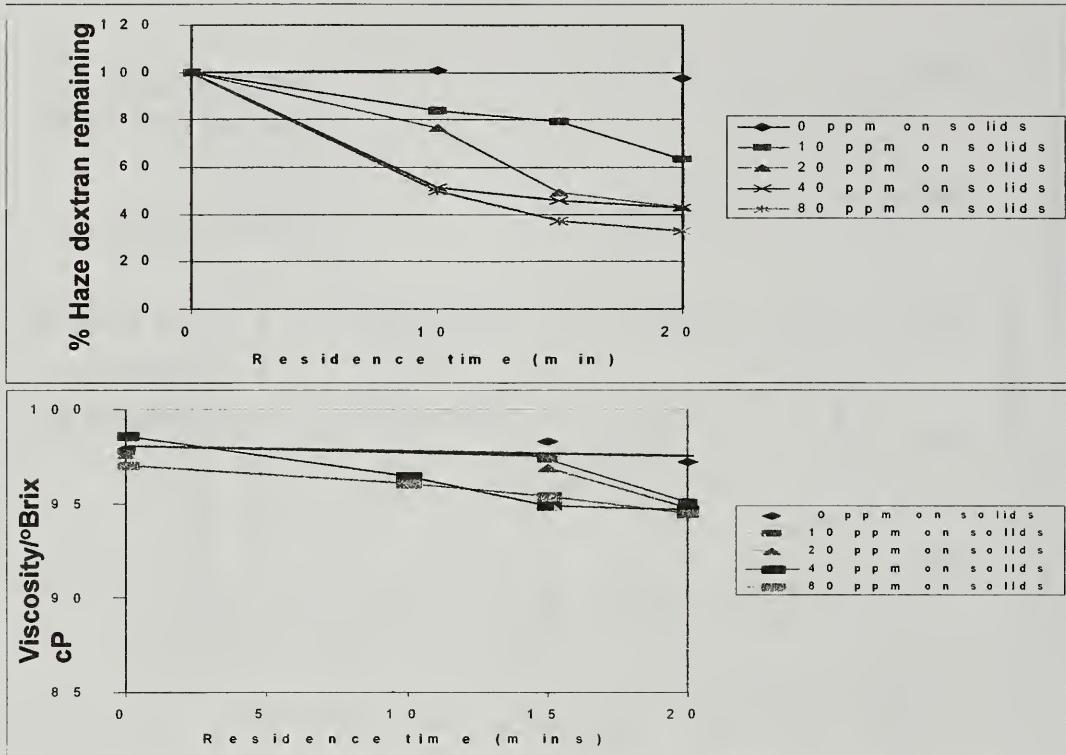


Fig. 5. Effect of various levels of "concentrated" dextranase in a final evaporator syrup (7230 ppm/°Brix Dextran) on (a) the removal of dextran and (b) reduction in viscosity. (Note: ppm/solids is equivalent to 4.5 x ppm/juice).

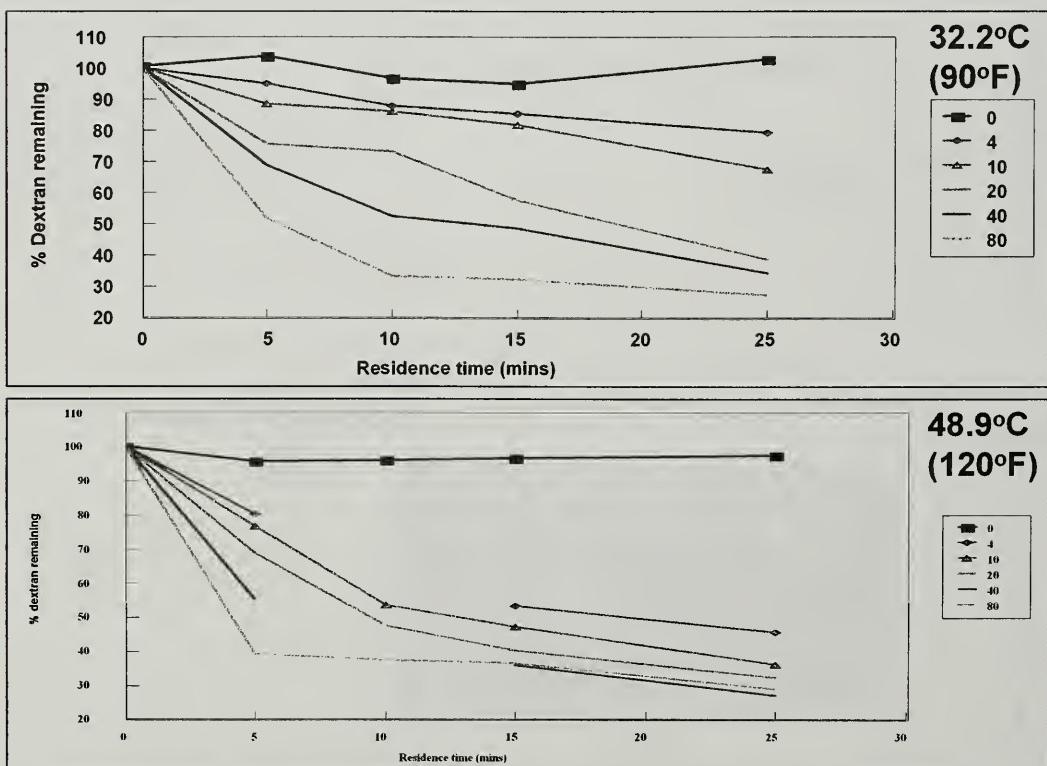


Fig. 6. Effect of various levels of "non-Concentrated" dextranase on removal of dextran from cane Juice (3380 ppm/°Brix). Application = ppm on juice.

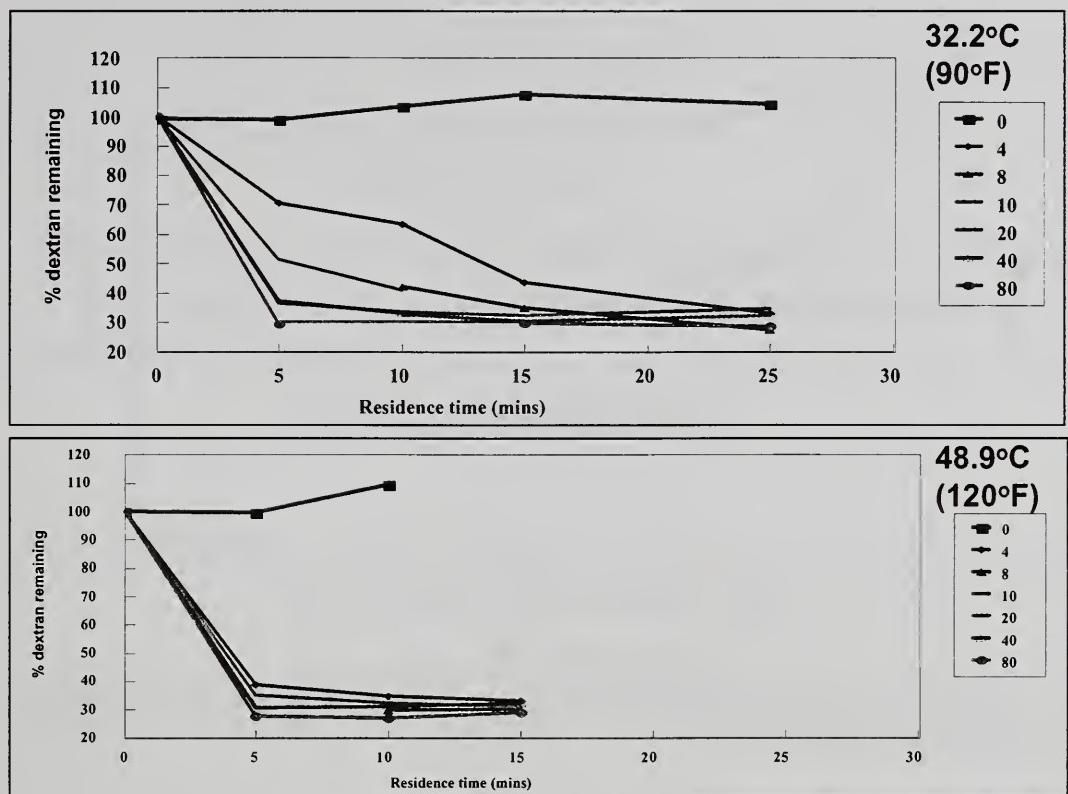


Fig. 7. Effect of various levels of "concentrated" dextranase on removal of dextran from cane Juice (3380 ppm/ $^{\circ}$ Brix). Application = ppm on juice.

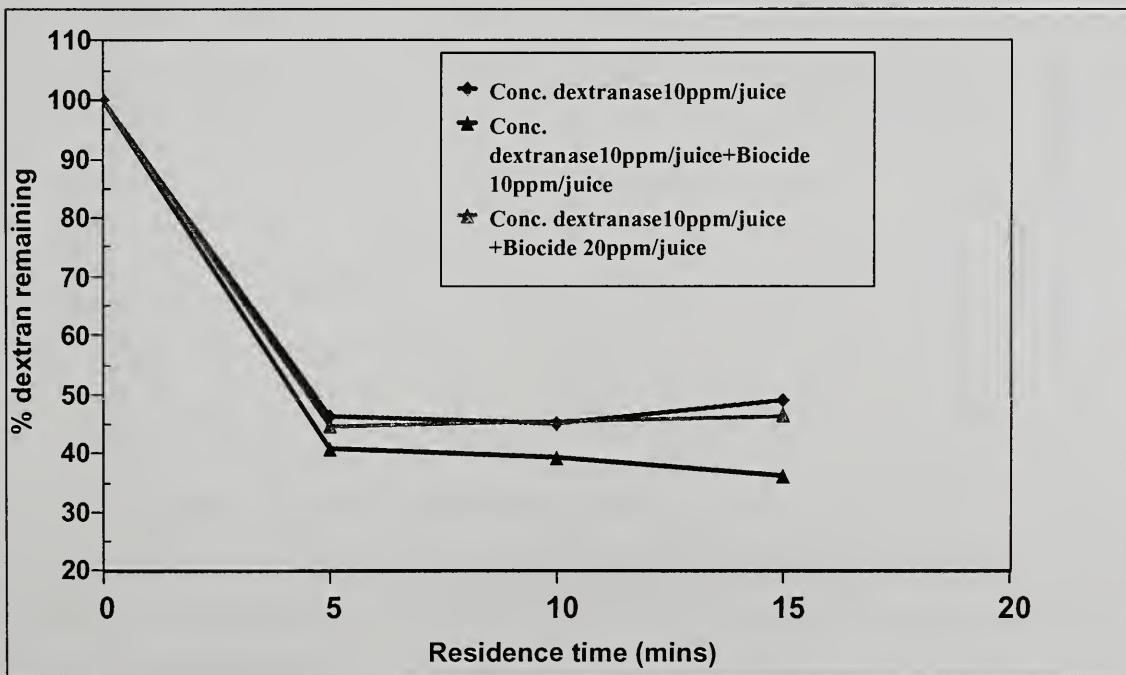


Fig. 8. Effect of Biocide (Carbamate) on dextranase action. (10 ppm dextranase on cane juice at 48.9°C (120°F) and pH 5.4).

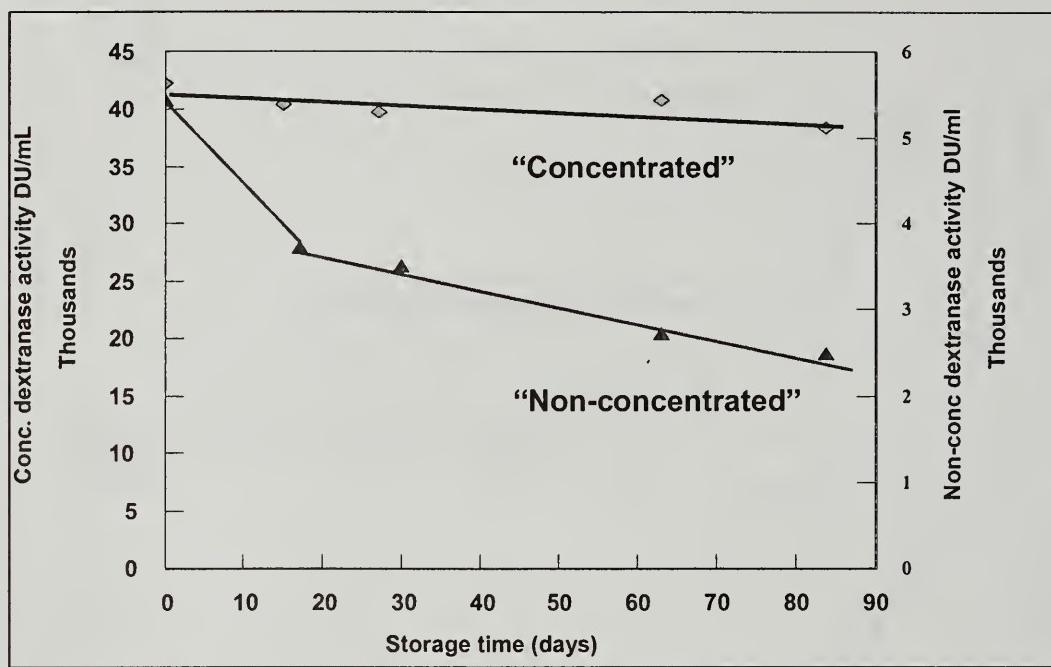


Fig. 9. Changes in activity of dextranases under simulated factory storage conditions. Stored at room temperature (~23-27°C) in shaded place over a 90 day grinding season.

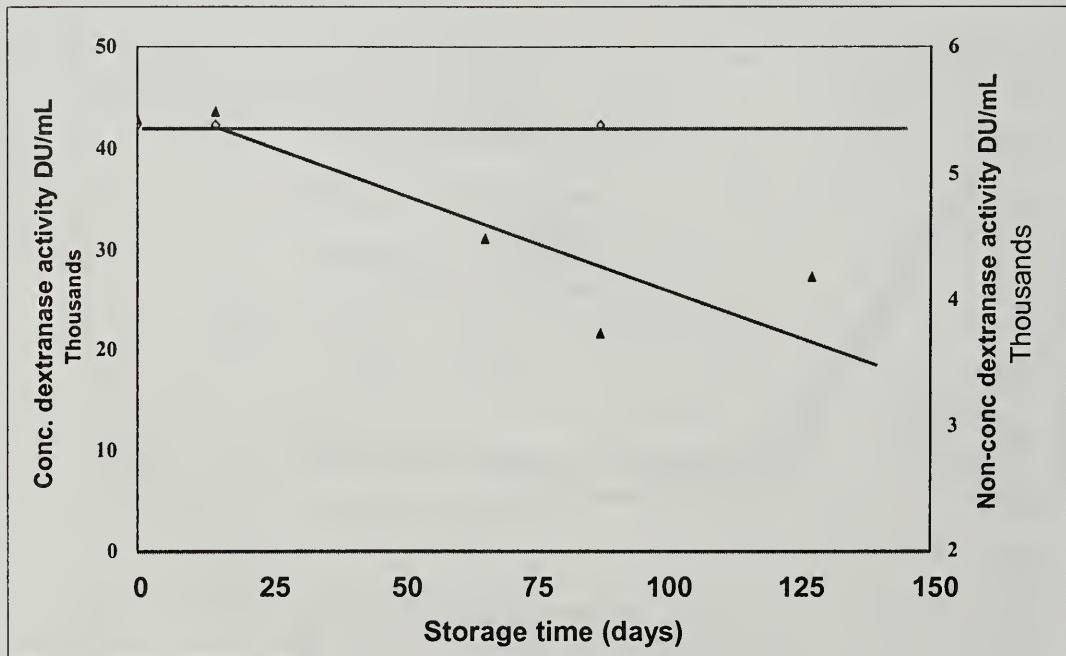


Fig. 10. Changes in activity of dextranases stored under refrigeration (4°C) over a grinding season period. Diamonds represent the "concentrated" dextranase, and triangles represent the "non-concentrated" dextranase.

DETERMINATION OF EXTRANEous MATTER AND ITS RELATIONSHIP TO DIFFERENT HARVESTING SYSTEMS IN THE COLOMBIAN SUGAR AGROINDUSTRY

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ABSTRACT

Extraneous matter content is one of the factors of great importance associated with the harvesting, loading and transport of sugarcane from the field to the mill. Extensive research conducted in different regions of the world has shown that extraneous matter affects both production and the quality of the end product (sugar). Consequently, one of the objectives proposed by the Colombian agroindustry is the need to have evaluation systems that guarantee the rapid and reliable determination of extraneous matter that enters a mill and to identify the best harvesting options, especially for green (i.e., nonburned) cane and to reduce the content of extraneous matter in the cane in the field or delivered to the mill. The implementation of sampling systems and control of extraneous matter by means of mechanized core sampling or the manual cleaning of samples obtained at a station for the postharvest evaluation of cane were evaluated for different harvesting methods. Similarly, three harvesting systems for green cane were evaluated under the conditions of the Colombian sugar sector, and their impact on the incorporation of extraneous matter during the harvest as well as on factory yield were determined. Based on the results obtained, it was concluded that harvesting sugarcane with the manually cut, clean green cane procedure (System 1) contributed to reducing the levels of extraneous matter in the cane for milling and to increasing sugar yields. The possibility of using NIR (near infrared spectroscopy) to quantify extraneous matter incorporated in the cane during its cutting, loading, transporting and delivery to the mill is being explored in conjunction with a pilot mill in the Cauca Valley.

Keywords: Extraneous matter, harvesting systems, NIR, green cane, factory yield.

INTRODUCTION

Extraneous matter is defined as plant matter other than the cane stalks rich in sucrose, which together with mineral matter consisting of dirt and stones, reduces sucrose levels and interferes with the processing of the harvested cane. Plant matter trash consists of leaves, tops, stools, leaf sheaths, suckers, dry cane and weeds. It is generally accepted that the levels of extraneous matter can be attributed to the harvesting system, the loading of the cut cane and the variety.

In the case of the Colombian sugar sector, the levels of green or dry leaves, tops, suckers and dry cane can be attributed mainly to the harvesting system, the cutter's work and the operation of the harvesting machines.

Chemical analyses of the different plant matter components considered to be trash have shown that in some cases the contribution of sucrose % cane should also be taken into account (e.g., for the tops and suckers, where levels of sucrose % cane range from 2-10%), depending on the season, varieties and harvesting conditions (**Table 1**). Nevertheless, the incorporation of higher contents of other components such as polysaccharides, potassium salts and color precursors (polyphenols) in the tops and suckers during the harvesting and later processing in the mill versus those of clean stalks could reduce or otherwise affect the recoverable sugar and quality of the end product (sugar). Observations on a laboratory scale have also made it possible to determine that the leaves and dirt have the greatest impact on the sucrose and recoverable sugar contents, reducing the recoverable sugar from 0.20-0.30 units (%) for each 1% of these materials included during the harvesting process (**Table 2**).

Table 1. Characteristics of extraneous matter.

Plant Matter	Sucrose % cane	Fiber % cane
Tops	2 – 6	13 – 20
Suckers	3 – 10	10 – 17
Leaves	---	20 – 50

Table 2. Changes in the content of sucrose and recoverable sugar (% ERS) for different kinds of extraneous matter.

Matter	Sucrose % cane	ERS % cane¹
Clean cane	15.0	13.0
Tops (1%)	- 0.13	- 0.15
Suckers (1%)	- 0.05	- 0.07
Leaves (1%)	- 0.17	- 0.21
Dirt (1%)	- 0.22	- 0.30

¹ ERS % cane = sucrose % cane – b x nonsucrose % cane – c x fiber % cane,
where b = 0.5 and c = 0.057.

With regard to the impact of extraneous matter on the zero percentage levels of nonsucroses and fiber % cane, it was observed that in trials carried out with variety CC 85-92, both the soluble mineral matter (dirt) and the extraneous plant matter showed similar increases in the nonsucroses: 0.020 units for each 1% of dirt or plant matter. On the other hand, the mineral matter increased the fiber % cane in greater proportion in relation to the components of the extraneous plant matter: an increase of 0.80 in the fiber % cane for each 1% of dirt incorporated in the clean cane (Larrahondo, 1999).

METHODOLOGIES

Procedures for determining the amount of foreign matter

Visits made to the sugar mills in Colombia where control of foreign matter is done indicate that there are two sampling and evaluation systems: mechanized core and claw. During the visits and data gathering, the number of samples per day, the sample size (weight) and average cost were estimated for each method.

To observe the functioning of new methodologies for quantifying the foreign matter that enters the factory, the respective calibrations and validations were initiated, using NIR (near infrared) spectroscopy. For the NIR calibration, 500 g of de-fibered cane mixed with different percentages in weight of plant foreign matter (leaves, tops, suckers, etc) and mineral matter (1-20%) were used. For each treatment at least two replications were analyzed, and the NIR Foss 6500 equipment was used with readings with the reflectance detector in the range of 1100-2500 nm. In addition, conventional procedures described for NIR (McDonald-Lewis, 1992; Wetzel, 1983; Burns and Ciurczak, 1992) were used.

Impact of foreign matter associated with different harvesting systems

The Colombian sugar industry has increased the green cane harvest area in compliance with their commitment not to burn in restricted areas in line with the Government's clean-production regulations. At present green cane is being harvested both manually and using mechanization. In the case of the former, there are three options or procedures for the harvest, which is reflected in the levels of extraneous matter that arrive at the mill. In general the systems of manually cut green cane are classified as the conventional system in green cane (no cleaning – System 2) and clean green cane (System 3).

From 1998 to 2002 commercial and noncommercial monitoring was done in the Colombian sugar mills, where the levels of foreign matter and sugar yields were determined for manual systems (2 & 3) as well as for mechanical harvesting (burned and green – Systems 4 & 5, respectively). Similarly, for Systems 2 and 3, comparisons were made with respect to the efficiency of the cane cutter, percentages of foreign matter and yield.

At CENICAÑA, the high leaf-shedding varieties (CC SP 89-1997, CC 91-1999, CC 89-2000 and MZC 74-275, the check) were evaluated at different sugar mills in order to determine the differences in sucrose % cane between the two manual systems (2 & 3).

RESULTS AND CONCLUSIONS

Procedures for determining the amount of extraneous matter

A state-of-the-art diagnosis regarding the control of extraneous matter in the Colombian sugar sector identified two sampling and evaluation systems: mechanized core sampling and the use of a manual claw. The latter, which is used in eight mills, can characterize or analyze a maximum of only 55 samples per day. On the other hand, when using the former system, which is used in four Colombian mills, from 115-120 evaluations are done daily with an average sample weight per evaluation of 8.5 kg (**Table 3**). In a series of simultaneous sampling and analyses (in duplicate) using the two systems for cane harvested manually and mechanized, corresponding to the commercial lots of 10 varieties, there were differences in determining the plant matter content incorporated during both the manual and mechanized cuts (**Table 4**); however, no significant differences were found for the overall evaluation of extraneous matter for the cane harvested manually. In general it was observed that the manual claw procedure gave the highest values of total extraneous matter, with a lower value of replication than for the mechanized core sampling (**Table 5**).

Table 3. Current systems for sampling extraneous matter in the Cauca Valley (Colombia).

System	No. Mills	No. Samples/Day	Average Sample Wt (kg)
Mechanized core sampling	4	115-120	8.5
Manual claw	8	10 - 55	350

Table 4. Comparison of two procedures for evaluating extraneous matter.

Type of Harvest	Difference Plant Matter ¹	Difference Mineral Matter	Total Difference
Manual	-1.2 (S) ²	2.2 (NS)	1.0 (NS)
Mechanized	3.9 (S)	1.6 (NS)	5.1 (S)

¹ Difference in the values of extraneous matter determined via the manual claw and mechanized core sampling.

² S = Significant difference at 95% confidence limit.

NS = Nonsignificant difference at a 95% confidence limit.

Table 5. Replicability of two procedures for evaluating extraneous matter.

Method	Replicability		
	Plant Matter	Mineral Matter	Total Extraneous Matter
Manual claw	5.9	17.1	13.1
Mechanized core sampling	5.4	20.9	22.7

Although the claw sampling procedure gives a good quantification and characterization of the extraneous matter, it is inconvenient because it is costly, requiring a greater number of workdays (average 5 per shift) in order to evaluate at least 10 commercial lots per day with a good level of replicability. As for the mechanized core sampling system, it requires fewer laborers (2 per shift) and can handle a higher number of samples, but its replicability is not so good as for the claw procedure (**Table 5**). Given the foregoing, the replicability of these two procedures will have to be improved, or studies will have to be conducted to determine how to reduce the costs of these evaluations while maintaining a highly reliable method for a large number of samples.

NIR spectroscopy has arisen recently as a new and better alternative for evaluating and quantifying the extraneous matter content that enters a mill. This methodology is based on a sampling of de-fibered cane that is taken before the first milling. The results reported by Brotherton and Berding (1995) and Staunton et al. (1999) in Australia, as well as the NIR calibrations carried out recently at CENICAÑA (Larrañedo, 2001), using the mechanized core sampling system with manual cleaning as the primary method indicate that this spectroscopic method has good potential (**Table 6**).

Table 6. Results of the calibration and validation for determining extraneous matter content.

Determination (%)	Range (%)	Method	Calibration		Validation	
			R	SEC	R	SEP
Clean cane	0 – 61	PLSR 2 nd deriv.	0.93	3.3	0.84	3.9
Extraneous plant matter	0 - 24	PLSR 2 nd deriv.	0.93	2.4	0.80	2.4
Extraneous mineral matter	0 – 15	PLSR 2 nd deriv.	0.92	1.7	0.86	2.1
Total extraneous matter	0 – 39	PLSR 2 nd deriv.	0.93	3.3	0.85	4.1

Impact of foreign matter associated with different harvesting systems

With the manual cutting the mills normally register levels of extraneous matter from 1.8 – 3.7% in burnt cane (system 1); from 2.7.7.7% in non-cleaned green cane (system 2); and from 1.5-3.0% in the clean green cane (system 3). When the harvest is mechanized, extraneous matter is around 9.5% in burnt cane (system 4) and over 10% in green cane (system 5). The data also indicate higher yields in sugar with the manual, clean green harvest as compared to the other options (**Table 7**) (Villegas, 2002).

Table 7. Extraneous matter and yield in sugar (% cane) by harvest system and procedure (nine mills)

Variable	System 1	System 2	System 3	System 4	System 5
Extraneous matter	3.3	5.9	2.6	9.5	12.4
Sugar yield (%)	11.8	11.1	12.1	11.6	10.9

System 1: Manual harvest – Burnt cane

System 2: Manual harvest – Green cane

System 3: Manual harvest – Clean green cane

System 4: Mechanized harvest – Burnt cane

System 5: Mechanized harvest – Green cane

In the manual harvests the difference in yield in favor of the clean green cane is approximately 0.5 and 0.8 percentage points as compared to the burnt cane. Similarly, the yields with clean green cane can reach 1.5 percentage points higher than with non-cleaned green cane of the mechanized harvest.

The commercial data on the cane harvested at a Colombian sugar mill during 1998 (**Table 8**) confirmed these results and showed that in the clean green cane, extraneous matter was reduced to 1% versus 6% for the conventional green cane harvest and 10% for the mechanized green. The commercial yield of sugar with the cut clean green cane was 2 points higher than the mechanized green and the conventional manual green harvest (Viveros et al., 1999).

Table 8. Comparison of the different harvesting systems in green cane (1998).

Variable	System 5	System 2	System 3
Average cutter efficiency (t/man/day)	---	3.0	1.5
Extraneous matter (%)	10.3	6.0	1.0
Sugar yield (%)	10.3	10.0	12.0

In System 3 the cutter removes the leaves and cuts the stalk at the base, removes the top and stacks the cane in the field. He also carries out a complete cleaning of the piling/loading zone. Thus this harvesting system has a higher cost than System 2, which could be a limitation for its adoption. To reduce this extra cost, it is necessary to increase the cutter's efficiency through actions that facilitate his work such as planting varieties that shed leaves, where it is easy to remove the leaves attached to the stalks and that are resistant to lodging. CENICAÑA varieties selected for self-trashing was found, for example, that variety CCSP 89-1997 in the procedure for System 3 had an efficiency of 3.2 t/man per day, while variety CC 89-2000 had a yield of 2.5 t/day, for an efficiency 3.9% higher than the average obtained with the commercial varieties for System 3. Similarly, among the varieties evaluated, the difference in sucrose (% cane) ranged from 0.4-2.1 percentage points in favor of System 3 (**Table 9**).

Table 9. Differences in sucrose (% cane) from clean green cane (System 3) and conventional green (System 2) harvesting systems for different varieties in Colombia.

Variety	Sucrose (%)¹			
	Mill A	Mill B	Mill C	Mill D
CCSP 89 - 1997	1.7	0.4	1.7	0.8
CC 91 - 1999	2.1	1.4	0.5	1.7
CC 89 - 2000	0.7	0.8	1.3	0.9
MZC 74-275	1.7	1.5	0.6	1.0
Average (X)	1.5	1.1	1.0	1.2

¹ Differences in sucrose from Systems 2 & 3 in favor of the latter.

CONCLUSIONS

- Extraneous matter per day of milling is higher for Systems 5 & 2, which suggests that for the sugar industry, System 3 is an alternative that can reduce the difficulties of milling and the sucrose losses in the mill.
- Despite the fact that System 3 requires more cutters, this can represent a socio-economic benefit by providing work for nonqualified labor.
- With respect to the R&D needs, the demand for varieties that facilitate the harvesting of green cane, both manually and mechanized, should be one of the objectives of a breeding program for supplying cane of optimum quality to the factories. The selection of self-trashing varieties not only greatly favors harvesting system 3 but also increases the efficiency of the cutter per day.

- The establishment of a standardized methodology for determining extraneous matter in the cane to be milled, with good precision and a larger number of analyses per day is another of the demands for R&D. At present NIR spectroscopy is a good technological option, which is in the process of development, commercial validation and adoption.

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MOLECULAR PROBES FOR ASSESSING BOILING DIFFICULTIES

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ABSTRACT

Louisiana sugar cane mills sometimes experience a phenomenon known as “hard to boil” massecuite (HTB). During the 2002 grinding season, two hurricanes and persistent rains caused a difficult harvest season, and episodes of difficult-to-boil and slow-to-boil episodes were experienced.

Trends observed in the HTB samples included higher levels of polysaccharides, dextran and calcium, as well as lower phosphate and invert. Polysaccharides, starch and dextran did not seem to be the main cause of the problem. Indications were that excess calcium may play an important role. The need to use extra lime for clarification due to high mud and polysaccharide levels, along with low natural phosphate in the juice, led to high levels of calcium carrying through the process, with a deleterious effect on crystallization.

In this study, a series of specific compounds were used as molecular probes to determine the causes of boiling difficulties, with an eye to improving the boiling characteristics of A-molasses, assuming that if a probe improved boiling, it could indicate the cause of the difficulty. All the probes improved boiling in some, but not all, of the samples.

INTRODUCTION

In the last several years, mills in Louisiana have experienced massecuites that will not boil, leading to low sugar recovery and excess molasses production. The problems are intermittent, usually occurring near the end of the season, after heavy rains or tropical storms, and sometimes after a freeze. These massecuites are often claimed to be “gummy” but also sometimes have little or no measurable dextran. In some cases, dextranase enzyme has been used. Although this is an important

problem for those mills that experience it, most of the information that exists about the phenomenon is anecdotal. When the problem occurs, there is little a mill can do to correct it. There are several accounts of things that work, but none have been clearly corroborated for the Louisiana situation. Among these are soda ash⁽¹⁾ and sodium hydrosulfite.

Soda ash. Soda ash (sodium carbonate, carbonate of soda, Na_2CO_3) is used in the beet industry in the main limers when the beets are deteriorated because of its effect on calcium solubility -- it both reduces the juice calcium and increases alkalinity.⁽²⁾ Calcium salts are reported to cause difficulties in boiling beet juices, and addition of soda ash is routinely recommended in the European beet industry.⁽³⁾ Addition of soda ash is more effective when the amount of lime used is quite high, as in the beet industry, and drops off as lime salts content decreases.⁽⁴⁾ This may explain why soda ash is sometimes effective in cane processing and sometimes not, as it would relate to the condition of the juice and the amount of lime used.

Further documentation on the use of soda ash appears in Appendix I, taken from an early edition of the Cane Sugar Handbook.⁽⁵⁾

In one excerpt from the 1917 edition of the Cane Sugar Handbook,⁽⁵⁾ “*S. S. Peck advises the addition of sodium carbonate to a raw juice that contains excessive quantities of sulphate which tend to form calcium sulphate scales in the heater and evaporators. The sodium carbonate decreases the amount of insoluble ash, phosphoric acid and lime in the filtered juice; it partially removes magnesia; it increases the amount of material removed by the filter presses and effects a slight increase in the proportion of organic impurities removed from the juice. The use of carbonate of soda materially increases the cost of defecation.*”

Sodium hydrosulfite. Sodium hydrosulfite ($\text{Na}_2\text{S}_2\text{O}_4$), also known as sodium dithionite or blankite, has been applied in the South African industry when massecuites are difficult to process; it is also used as a bleaching agent in sugar production in China. It has been found to significantly reduce massecuite viscosity.^(6, 7) A study in 1978 found that sodium hydrosulfite used as a pan aid showed improvements in boiling time, exhaustion, centrifugal capacity and final molasses purity.⁽⁸⁾

Melassigenic compounds. Melassigenic compound are impurities found in massecuites that increase sucrose solubility and therefore carry sucrose into molasses, slowing crystallization in the process, and lowering yield. The melassigenic coefficient is defined as the number of parts of sucrose that will be taken into molasses at saturation, per part of nonsucrose.^(9, 10) Among the most melassigenic compounds are cations, with potassium salts having the highest coefficients, in the order: K > Na > Ca > Mg. Potassium and sodium salts of lactic and acetic acid are especially melassigenic.

Polysaccharides and other impurities. The significant polysaccharides in cane processing are dextran, starch, and soluble cell wall polysaccharide, a group of polysaccharides collectively known as indigenous sugarcane polysaccharide (ISP). Dextran and starch greatly increase viscosity and hinder crystallization. It is possible that polysaccharides may be melassigenic. Numerous studies have shown the effect of polysaccharides and other impurities on sucrose crystallization kinetics (rate of crystallization) and their effect on the morphology of the crystal. Compounds shown to have deleterious effects on either or both, include raffinose, found mainly in beet sugar processing,⁽¹¹⁾

theanderose, an oligosaccharide found in cane sugar processing,⁽¹²⁾ cane juice fructans⁽¹³⁾, beet coloring matter,⁽¹⁴⁾ glucose and fructose,⁽¹⁵⁾ and various deteriorated cane oligosaccharides and polysaccharides.^(16, 17, 18)

Heat transfer. Another factor that may cause HTB phenomenon is inhibition of heat transfer, caused by a fouling of the heating surfaces in evaporators⁽¹⁹⁾ or the production of small bubbles throughout the massecuite, which interferes with heat transfer.⁽²⁰⁾ Pamela Morel du Boil recently reported on the possibility that decarboxylation of aconitic acid could produce bubbles caused by CO₂,⁽²¹⁾ thereby slowing heat transfer and crystallization.

Additives as molecular probes. Based on the survey results of syrups and molasses composition, which are presented here in summary, it seemed reasonable that there may be several causes for HTB phenomenon. Several compounds were chosen as molecular probes for exploring causes and possible solutions of HTB. The compounds chosen each has a different mode of activity: Hydrogen peroxide(H₂O₂), a strong oxidizing agent; ammonium bisulfite (ABS), a strong reducing agent and supposedly able to reduce viscosity; SDS (sodium dodecyl sulfate, sodium laurel sulfate), an anionic surfactant; urea, a chaotropic agent that can break up hydrogen bonds; EDTA, a calcium chelator; soda ash, for its activity with calcium, as described in the Introduction. Subsequent to a report by Saska,⁽²²⁾ hydrochloric acid was also investigated. The activities and commercial uses of these compounds are summarized in Table 1.

Table 1. Additives used as molecular probes in boiling experiments.

Process Aid	Functions
H ₂ O ₂	Strong oxidizer; bleaching agent; approved for food use. Does not affect polysaccharides.
ABS	Strong reducing agent; antimicrobial, oxygen scavenger; bleach, breaks up polysaccharides; slightly lowers viscosity; used extensively in beet sugar processing.
SDS	Detergent, anionic surfactant; lowers surface tension of solutions; emulsifies fats; disaggregates protein and polysaccharide complexes. Used for the isolation of proteins; used in cosmetics; used in washing powders, toothpaste, shampoo, etc.
Urea	Chaotropic agent; breaks up hydrogen bonds; disaggregates proteins.
EDTA	Calcium and heavy metal chelator; breaks calcium complexes by binding calcium; keeps calcium in solution; prevents calcium scale; used to remove calcium scale (ie, commercial product example is Dow's Versene). Approved for many food uses; used in pharmaceuticals and food processing
Soda Ash	Increases the reactivity of lime through a common ion effect. Used to help process deteriorated sugar beets.
HCl	Acidifier, may lower viscosity; action in boiling phenomena not known.

EXPERIMENTAL

Composite 6-hour samples of A-molasses were gathered over the 2002-2003 grinding season from a Louisiana mill. A-molasses was chosen because this is where the problem is most likely to be manifested. According to factory personnel, the HTB phenomenon is characterized by the following: The sugar will not crystallize, the syrup "does not move," maximum temperature in the pan drops from 155°F to 130°F. When the problem occurs, neither surfactants nor the addition of soda ash have had any effect. Other samples of HTB syrup were analyzed from other mills, whenever they became available.

Samples were analyzed for sucrose, glucose, fructose, ash, pH, brix, calcium, phosphate, oligosaccharides, total polysaccharides, dextran, starch and lactic acid. The indigenous sugarcane polysaccharide (ISP) was determined as the difference remaining after the sum of starch and dextran was subtracted from the total polysaccharide value.

In order to visualize the effect of each probe, the "boiling profiles" of treated and untreated solutions were compared. These profiles were established by recording the amount of condensate collected over time while boiling under a constant vacuum of 22in/Hg. The solutions were boiled in a 500 ml reaction vessel fitted with a condenser, magnetic stirrer and thermometer, set in a hot water bath. The condensate was collected in a graduated cylinder. The entire installation was insulated. Vacuum was supplied by an oil pump. Experiments were conducted with 200 g of product at 50 brix with 1000 ppm of the molecular probe added.

Samples consisted of Mix #1, a composite of several well-boiling A-molasses; Mix # 2, a composite of HTB samples from an episode that took place October 23-25, 2002; and an example of poor boiling evaporator syrup (St. Mary).

RESULTS AND DISCUSSION

Summary of A-Molasses Composition

Table 2 summarizes the average results of twenty normal boiling samples of A-molasses, from Sept. 23 to Oct. 20, 2002; and four HTB samples from October 23 and 25, 2002. The data show 1.5 times more polysaccharide and 2.2 times more dextran in the difficult samples than in the normal samples. The starch variation (not shown) was within the normal range seen in Louisiana and is affected by amylase usage. Starch was not considered to be a factor. Calcium did not show any significant differences, but the phosphate in well boiling was twice that of the poor boiling samples.

Table 3 summarizes another set from the same mill comparing two good samples from November 20, 2002, and 5 HTB samples from December 4-8, 2002. This set was opposite to the results in Table 2, in that polysaccharides and dextran were in the normal range. The pH average was consistently higher in poor boiling samples, although the individual variation was high. Calcium was 27 % higher in the poor boiling samples and again the phosphate in well boiling was twice that of the poor boiling samples. Starch was not considered to be a factor.

Table 2. Summary of results, Sept. 23 to Oct. 20, 2002, Louisiana sugar mill

Sample description	pH	Total polys	Dextran	Ca	PO ₄
9/23-10/20, "normal," average, n = 20	6.16	10,251	3377	8279	298
10/23 and 10/25, "poor," average, n = 4	6.32	15,132	7327	7891	146
Ratio of poor/normal	1.03	1.48	2.17	0.95	0.49

- All results are in ppm on Brix refractometer solids

Table 3. Results from November and December samples

Sample description	pH	Total polys	Dextran	Ca	PO ₄
Good, Nov. 20, average, n = 2	6.03	11,450	3939	10,498	285
Poor, Dec. 4-8, average, n = 5	6.49	9,330	3198	13,328	127
Ratio of poor/normal	1.08	0.81	0.81	1.27	0.45

- All results are in ppm on Brix refractometer solids

Table 4 shows the data for evaporator syrups, one with "good" and one with "bad" boiling. "Bound calcium" refers to a phenomenon noted with the syrups in Table 4. When oxalic acid is added to a solution of molasses or syrup containing calcium, it will bind to the calcium to make insoluble calcium oxalate, which precipitates out. In the poor boiling sample in Table 4, addition of a small amount of oxalic acid immediately resulted in precipitation of calcium oxalate, whereas in the well boiling sample, there was a delay of about 10 minutes, and the precipitate formed slowly. The delay was felt to be due to the calcium being bound by components in the syrup, making it unavailable for other reactions. Further study should be done to determine if a predictive test can be developed.

Table 4. Comparison of evaporator syrups with different boiling characteristics.

Syrup	% Lactic acid	% Ca	% PO ₄	Bound Ca	Total Polys
Good	0.026	0.179	0.018	Yes	4040
Bad	0.040	0.315	0.008	No	6253
Bad/Good	1.54	1.76	0.44	---	1.55

The results of analyses on two evaporator syrups with different boiling characteristics are shown in Table 5. Differences are again noted in the concentration of calcium (a two-fold increase in the HTB sample), polysaccharides and dextran. The differences in polysaccharides and dextran are not considered significant. Higher starch in the well-boiling sample indicates that starch was not a factor in this HTB phenomenon.

Table 5. Comparison of evaporator syrups with different boiling characteristics. (Results are based on solids.)

Sample	% Sucrose	% Invert	% Calcium	Polys, ppm	Dextran, ppm	Starch, ppm
Good	75.6	6.4	0.51	10,100	3,400	2,700
Bad	72.3	9.3	0.98	12,400	5,000	1,600

Table 6 shows the average values for 5 well boiling and 9 poor boiling A-molasses over the 2002 season for a range of constituents.

Table 6. Comparison of constituents in A-molasses from a Louisiana mill: Well boiling vs poor boiling. Well boiling = 5 samples; poor boiling = 9 samples.

Type	Glc	Fru	Invert	G/F	Ash	Ca	PO ₄	Lactic	Polys
Good	5.40	5.98	11.38	0.91	7.44	8279	298	845	9943
Bad	4.37	5.20	9.57	0.84	7.55	11,458	150	1144	11,582

Table 6. Comparison of constituents in A-molasses from a Louisiana mill: Well boiling vs poor boiling. Well boiling = 5 samples; poor boiling = 9 samples.

Type	pH	Dextran	Starch	ISP	Sucrose
Good	6.10	3250	1784	5090	70.16
Bad	6.39	4638	1056	5733	71.02

Several trends are evident in the results in Table 6. The poor boiling samples were higher in calcium, polysaccharides, dextran and lactic acid. Phosphate was considerably lower, by half. The pH was higher in the HTB samples, another consistent trend noted in the industry. This probably indicates that volatile acids are being formed and then evaporated off, allowing the pH to rise. The formation of acids may take place at the expense of the formation of dextran, explaining, in part, why dextran is often not elevated in some HTB samples, and also explaining the higher lactic acid results. The results also indicate that polysaccharides, including starch and dextran, are not significantly different in either good or bad samples. However, slightly elevated starch and/or dextran may provide some synergistic effects in HTB.

Of interest are the observations about the invert levels and the glucose/fructose (G/F) ratios. Both invert and G/F ratios were lower in the poor boiling materials. Invert sugar decreases the solubility of sucrose, and therefore, can aid in crystallization (up to a point). A lower invert could indicate destruction of invert, either through bacterial action or by alkaline degradation. (Salts of invert degradation are moderately melassigenic.)^(9,10) G/F ratios usually drop during processing, indicating a more rapid loss/destruction of glucose than fructose.

Boiling Experiments Using Molecular Probes

Figure 1 compares the boiling profiles of pure sucrose and Mix #1. While Mix #1 was considered a well-boiling mixture, the level of impurities has impacted the boiling properties compared to pure sucrose.

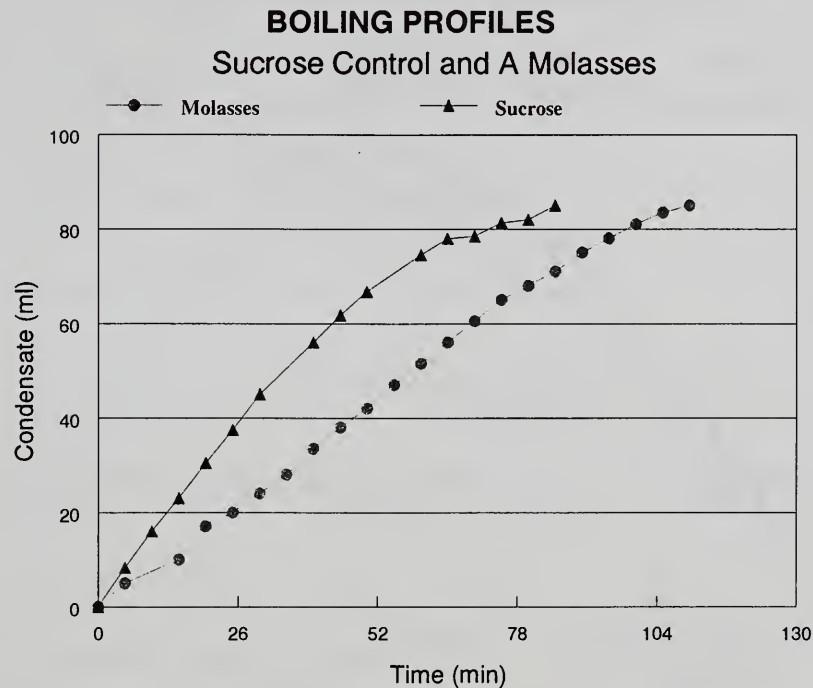


Figure 1. Boiling profiles of pure sucrose and well-boiling A-molasses.

Figures 2, 3 and 4 show the effect of various probes on the boiling profiles of Mix #1 (well boiling), Mix #2 (poor boiling), and St. Mary syrup (poor boiling). Most of the additives showed, in general, improvements in boiling of Mix #1 and St. Mary evaporator syrup, but had little effect on Mix #2, a poor boiling sample.

Variable effect of soda ash on boiling profiles. The effect of soda ash was variable. In Mix #1 (Figure 2), it had a very good effect, but in the St. Mary syrup, it had no effect at all (Figure 4). Unfortunately, there was not enough of Mix #2 to test the effect of soda ash. These results again point to the possibility that different mechanisms are responsible for HTB.

Over-all, hydrogen peroxide consistently improved boiling the most in the three sets of samples. SDS, a surfactant, had the best effect on Mix #1, but little effect on the other two. The qualitative effects of the probes are summarized below.

Mix #1: $\text{H}_2\text{O}_2 = \text{SDS} = \text{Soda Ash} > \text{EDTA} = \text{Urea} > \text{ABS} > \text{Untreated Molasses}$

Mix #2: (Additives had little effect): $\text{H}_2\text{O}_2 \geq \text{ABS} = \text{SDS} = \text{EDTA} = \text{Urea} \geq \text{Untreated Molasses}$

St. Mary Evaporator Syrup: $\text{H}_2\text{O}_2 > \text{Urea} > \text{ABS} > \text{EDTA} = \text{SDS} > \text{Soda Ash} = \text{Untreated Syrup}$

The temperature profiles also showed some differences, with lower maximum temperature being reached by the untreated samples than in the effectively treated samples.

A Molasses Mix #1, Boiling Profiles

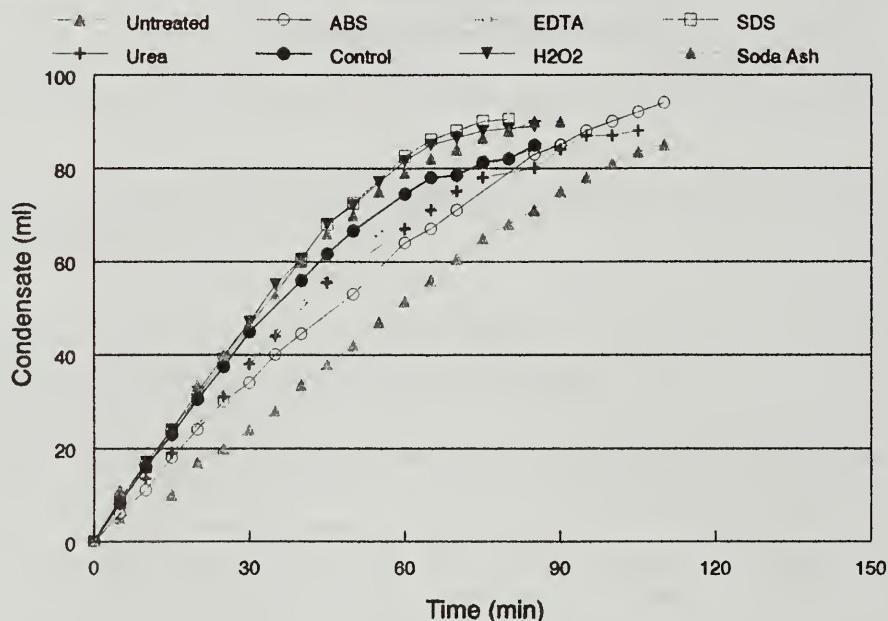


Figure 2. Effect of additives on the boiling profile of Molasses Mix #1 (well boiling).

A-Molasses Mix #2, Boiling Profiles

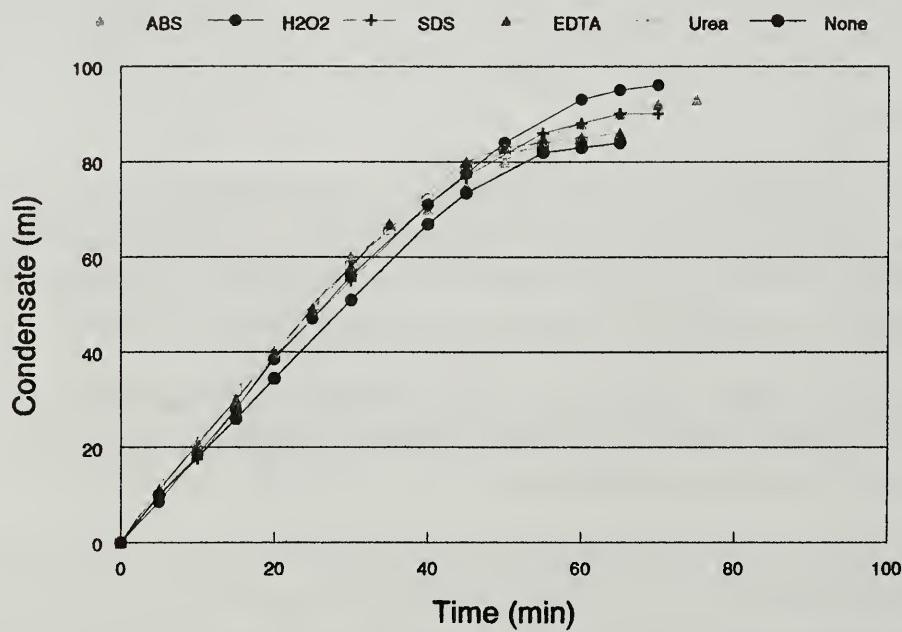


Figure 3. Effect of additives on the boiling profile of Mix #2 (poor boiling).

St. Mary Syrup Boiling Profile

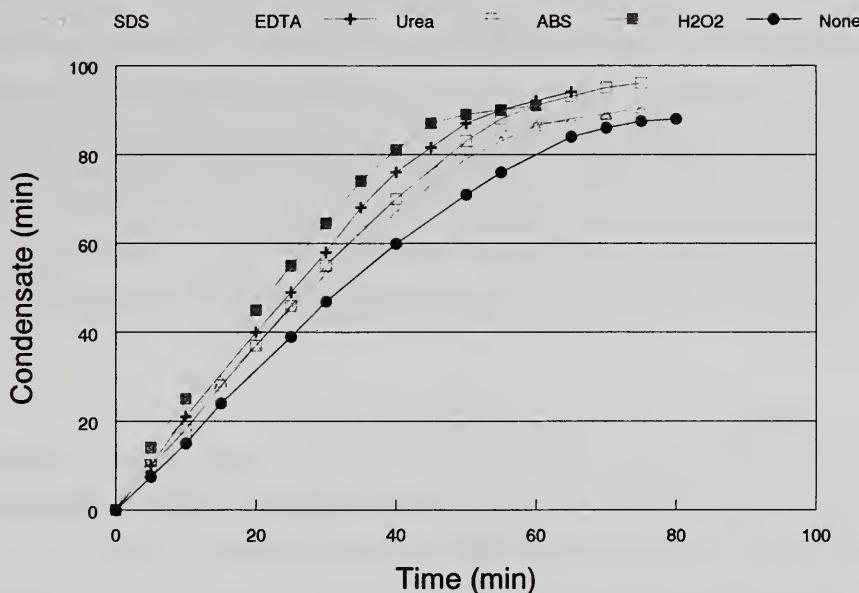


Figure 4. Effect of additives on the boiling profile of St. Mary evaporator syrup (poor boiling).

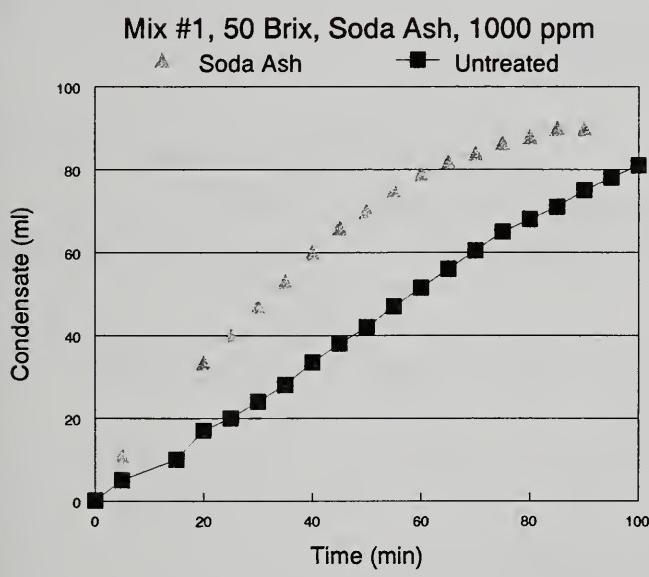


Figure 5. Effect of soda ash on boiling profile of Mix #1. (Strong effect.)

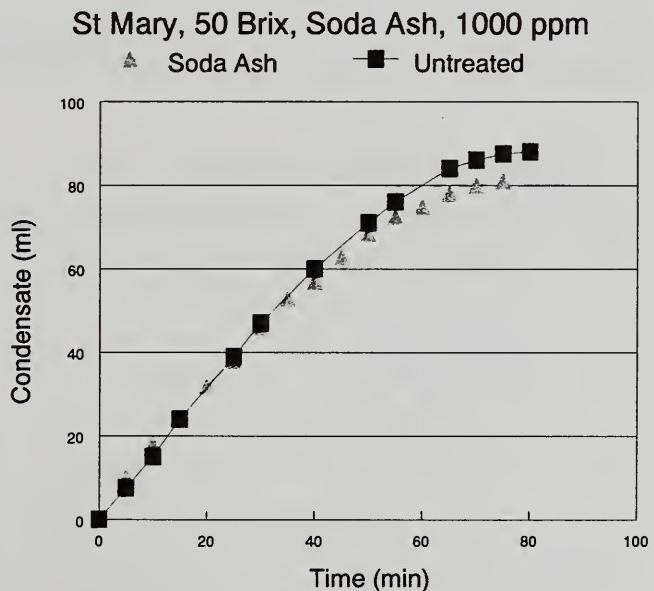


Figure 6. Effect of soda ash on boiling profile of St. Mary evaporator syrup. (No effect.)

Effect of Additives on Polysaccharides

In earlier work, we had noted that treatment of cane juice samples with ammonium bisulfite (ABS) resulted in apparent destruction of polysaccharides. In the present experiments, the effect of the additives (with the exception of soda ash) on total polysaccharides, dextran and starch was determined. The results showed that the action of ABS was greatest against starch and dextran, removing 20-30% of each. Urea also resulted in lower starch and dextran values. However, ABS treatment resulted in solutions that were harder to filter and they seemed to develop a precipitate. The amount added, 1000 ppm, was high and not optimized, so it could be that ABS in a lower concentration might be more effective. Hydrogen peroxide had no effect on polysaccharides, nor did urea, SDS or EDTA.

Effect of Acid on Boiling

As mentioned above, based on reports of hydrochloric acid causing improved boiling,⁽²²⁾ we decided to investigate it. Willems⁽²³⁾ recently reported on the addition of large amounts (5 T/day) of sulfuric acid into beet thick juice to improve crystallization and purity.

Poor boiling A-molasses #459 and poor boiling evaporator syrup from St. Mary were adjusted to pH 5.0 with HCl and a comparison of boiling profiles was obtained. The results are shown in Figure 7 for A-molasses and in Figure 8 for the evaporator syrup. Boiling was improved in the A-molasses but there was no effect on the evaporator syrup. The sugar analysis is shown in Table 7. There was no loss of sucrose, although the G/F ratio would indicate some Maillard reaction going on with consumption of glucose, and a slight increase in invert. Loss of sucrose under acidic conditions is a major concern, but these results show that the effects could be minor.

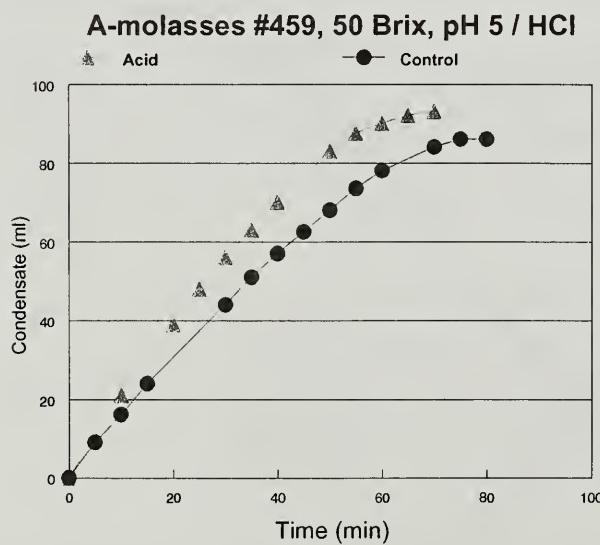


Figure 8. Effect of boiling A-molasses with HCl, at pH 5.

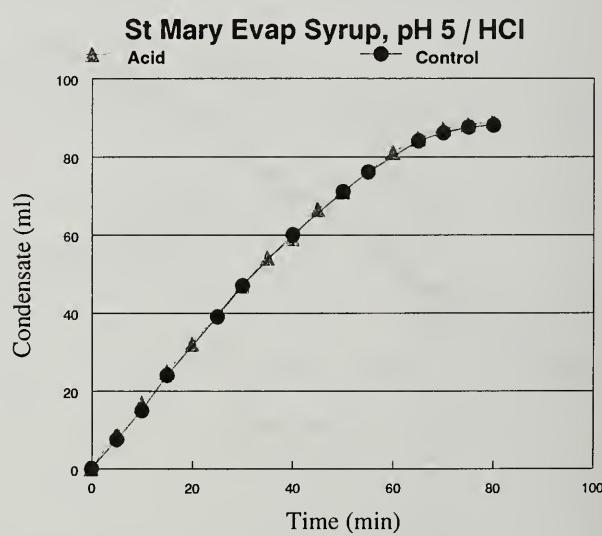


Figure 9. Effect of boiling evaporator syrup with HCl, at pH 5.

Table 7. Effect of hydrochloric acid addition on sucrose in boiling syrup and molasses.

Sample	Sucrose	Glucose	Fructose	G/F	Invert
St. Mary evaporator syrup, feed	86.69	2.10	2.25	0.93	4.35
Evaporator syrup, after boiling	86.53	2.38	2.30	1.03	4.68
A-molasses, feed	63.91	5.48	6.12	0.89	11.60
A-molasses, after boiling	64.25	5.47	6.97	0.79	12.44

CONCLUSIONS

This study, and others, indicate that the hard-to-boil phenomenon may have different causes. The different causes for HTB, such as high calcium in syrup and molasses, high polysaccharide and dextran concentrations, or others, such as bubbles or formation of fouling surfaces, will explain why additives perform well in one situation and not in another. Most of the boiling probes improved boiling over the untreated massecuite. In this study, HCl and H₂O₂ were the most effective agents, and soda ash had variable efficacy. HCl improved boiling without causing sucrose loss.

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According to the handout, “The benefits of adding soda ash to the main limer seem to include increasing the reactivity of the milk of lime. This could be explained by a common ion effect. When processing beets in good condition, there is plenty of natural alkalinity -- an excess of sodium and potassium ions relative to the soluble anions present. However, once the beets have deteriorated, there is an excess of soluble anions relative to the alkali ions. This excess is either created or increased in the main limer where hydroxide reacts with invert and other neutral species to form organic acids. The creation of these acid anions consume hydroxide, leaving an excess of calcium ions. As the concentration of the calcium ions builds up, it suppresses the solubility of the calcium hydroxide. Thus, when processing deteriorated beets, the main limer has a lower hydroxide concentration just when a high concentration is most beneficial. Adding soda ash to the man limer instantaneously precipitates calcium as carbonate, thus shifting the equilibrium towards an increased hydroxide concentration. This would explain the apparent increased reactivity with more stable juices and decreased filter problems.”

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19. M. Saska, LSU. Saska recently reported that difficult boiling A-molasses forms a fouling layer or film on the heating surfaces of evaporators (ASSCT, Baton Rouge, Feb. 2003; ASI Factory Operations Seminar, April 2003), but that no differences in the composition of the fouling layer compared to the original massecuite could be determined.

20. Frank Cole, in discussion at ASSCT, Baton Rouge, Feb. 2003. Mr. Cole observed fine bubbles throughout some difficult boiling massecuites, which improved greatly upon addition of antifoam.

21. Morel du Boil, in a paper given in Brazil, 2003, information from Ben Legendre.

22. Saska, M. In a presentation at the Feb 2003 meeting of the American Society of Sugar Cane Technologists in Baton Rouge, Saska reported that addition of hydrochloric acid into A-molasses approximately to pH of 5, enhanced boiling characteristics.

23. Willem, M.L., Meet at the interface of “beet quality” and “sugar processing,” presentation at American Society of Beet Sugar Technologists meeting, San Antonio, TX, Feb 26-Mar 1, 2003.

APPENDIX 1

Notes on Soda Ash

The material below is quoted directly from the Spencer-Meade Cane Sugar Handbook, 7th Edition, 1917, p. 76:

“Juices which have begun to ferment are better neutralized with soda, rather than with lime, as the latter produces soluble lime salts that are very objectionable. The use of soda ash (carbonate of soda) to neutralize excess acidity in juice from burned cane is common practice in many factories. The soda salts are also useful in neutralizing molasses. The carbonate apparently has a beneficial effect when taken into the pan in boiling low-grade massecuites.

“Carbonate of soda is an incomplete precipitant of lime from its salts in sugar solutions. In a series of experiments, de Grobert found that the addition of carbonate of soda to a sirup in the proportion of its equivalent of lime precipitated about 52 percent of the lime. The addition of two equivalents more of soda precipitated 81 percent of the lime. A part of the soda remained in a free state, and the remainder was neutralized by the organic non-sugar. M. de Grobert states that if carbonate of soda is used in juices, sirups, etc., it should be the theoretical quantity required to combine with the lime.

“L. R. Cook cites an experience in pan-boiling in beet-sugar work when an excessive amount of lime salts in the low-grade material prevented the pan from boiling. Cottonseed oil was injected into the massecuite and improved the conditions. The oil was then saponified with caustic soda. This immediately further improved the conditions and the pan boiled freely.

“A viscous or very heavy massecuite that is difficult to pump usually yields readily after the addition of carbonate of soda. Such massecuites are more easily purged in the centrifugals after the soda treatment.

“S. S. Peck advises the addition of sodium carbonate to a raw juice that contains excessive quantities of sulphate which tend to form calcium sulphate scales in the heater and evaporators. The sodium carbonate decreases the amount of insoluble ash, phosphoric acid and lime in the filtered juice; it partially removes magnesia; it increases the amount of material removed by the filter presses and effects a slight increase in the proportion of organic impurities removed from the juice. The use of carbonate of soda materially increases the cost of defecation.”

THE SHAPE OF SUCROSE MOLECULES

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ABSTRACT

The shape properties of sucrose have many important ramifications. They are responsible for its sweet taste, for its crystallization behavior and for many of the intermolecular interactions that are unique to sucrose. The shapes of sucrose are conveniently described in terms of the extent of rotation of the glucose and fructose monomers about their bonds to the mutually held oxygen atom. The most definitive method for studying shape is diffraction crystallography. It precisely locates each of the atoms but it requires a crystal and in most cases gives only a single structure when the desired result is a set of probabilities for the range of plausible structures. The problem of a limited number of structures has been overcome by finding crystals of very similar molecules and complexes, giving a wide range of observed structures. Another precise method is to calculate the energy of the different shapes to learn the shape with lowest energy. In principle, that structure is the most likely one, and structures with progressively higher energy are progressively less probable. Although this method can give a very precise answer, it may not be accurate. There are many ways to calculate the energy and they give different answers. Our poster presents a method that shows all of the crystallographically observed structures to have fairly low energy. This suggests that other shapes are unlikely to be observed in future experiments. The lowest energy occurs when hydrogen bonds connect the glucose and fructose rings, but many of the observed structures do not have such hydrogen bonds. This suggests that hydrogen bonds do not determine the structure but that they can form if the molecule otherwise has the correct shape.

INTRODUCTION

This presentation is about the prediction of the shapes of sucrose molecules by both experimental and theoretical methods. The major shape variables are the linkage torsion angles ϕ and ψ , shown in Figure 1.

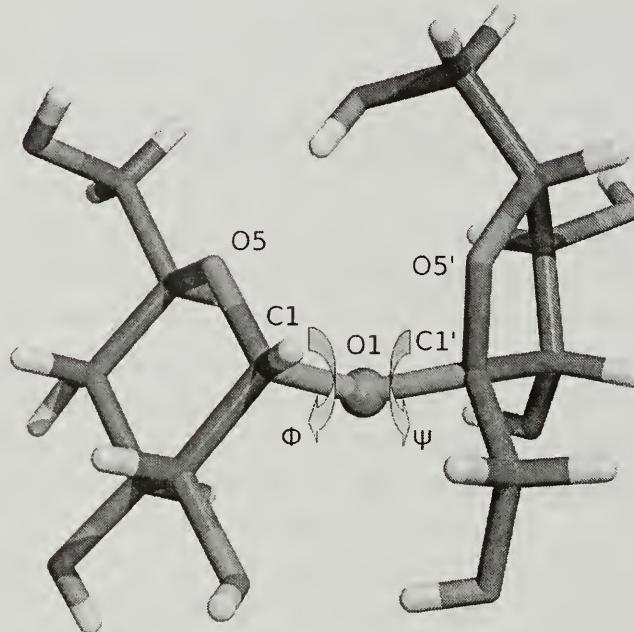


Figure 1. SUCROS01 crystal structure of sucrose showing the glycosidic linkage, ϕ and ψ .

A computational chemist systematically rotates the glucosyl and fructosyl rings about the C–O bonds of the linkage and calculates the energy at each increment of rotation. This energy indicates how much strain is added to the molecule by twisting it into that particular shape. A surface plot of energy vs. ϕ and ψ has hills and valleys (Figure 2).

Another representation of the same information is the contour plot, which we use below. In principle, the low energies on the ϕ , ψ plot, or map, correspond to the likely shapes of the molecule. The question is whether the map is accurate and predictive.

Crystallographers place crystals in an intense beam of very short wavelength radiation (e.g. x-rays) and precisely determine the positions of the atoms in the molecule. For a given molecule, identical results are expected for all crystals grown under the same conditions. Indeed, the sucrose structure has been reported about a dozen times, always with the same result! Because sucrose exists in other environments, e.g. solution, where the shape is not so easily determined, the relevance of its crystal structure is a major question. To answer that question, we also look at all crystal structures of molecules that are specifically related to sucrose. Each molecule will have a different environment in its crystal. If sufficient numbers of related molecules are analyzed, the resulting range of shapes should indicate the shapes to be expected for experiments that have not yet been carried out. This only works well if the intramolecular forces are relatively strong, and if the various environments perturb the structure in small, random ways.

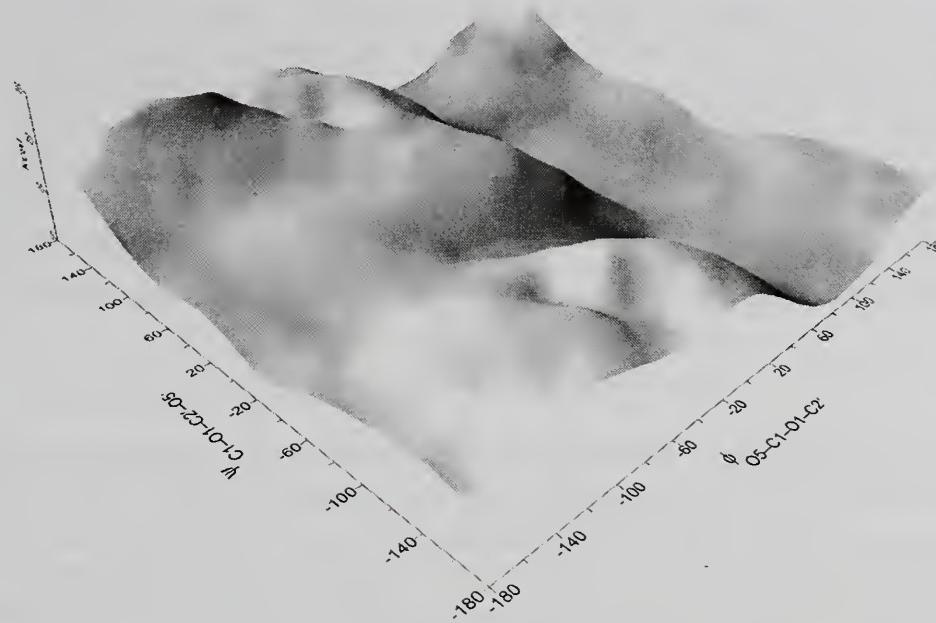


Figure 2. 3D ϕ, ψ surface map of the Me-THF-O-THP sucrose analog.

The present work combines crystallographic and theoretical studies for an enhanced view of likely molecular shapes. If shapes determined by experiment are predicted by the energy calculations, then it can be inferred that new structures will also fit into the low energy regions that might or might not be populated at present. Also, the factors that determine molecular shape can be clarified in such an analysis.

At a previous SPRI Conference in Georgia (Savannah, 1998), we showed a new energy map for sucrose. The energy was a hybrid of both empirical force field and quantum mechanics calculations. Empirical force fields are also called molecular mechanics (MM), and they are based on equations that describe, for example, the ideal length for a bond and the energy increases that result from stretching or compressing that bond. Quantum mechanics (QM) methods are also called electronic structure methods. Hybrid methods use a high-quality (such as QM) method for a small, critical part of the molecule and a lesser method (such as MM) for the remainder. We defined the small, critical part as the carbon and oxygen atoms of the rings and linkage. For sucrose, we have tetrahydrofuran and tetrahydropyran rings linked by oxygen. We denote this analog as THF-O-THP. Compared to earlier MM-only studies, the QM calculations in the hybrid method gave improved values of the energy for the stereo-electronically complex glycosidic linkage in sucrose.

Since 1998, the theoretical work has matured. Our work with the hybrid method has been published from several different perspectives (French et al., 2000a,b; 2001). In particular, the hybrid method was applied to numerous other disaccharides and it was always predictive. Also, an analog that had a methyl group attached to C2' (Me-THF-O-THP) gave a good accounting for the crystal structures, unlike the simpler THF-O-THP analog. Importantly, the relative HF/6-31G* energies are subject to only minor improvement by using higher levels of theory. Progress is also included on another problem in modeling carbohydrates. The calculated energy of

carbohydrates depends on the combinations of orientations of the rotatable OH and CH₂OH groups. If there were three-fold rotations for all 11 rotatable groups, there would be 177,147 possible combinations. Another 24 of the most likely combinations have now been included in the empirical force field calculations that are used in the hybrid method, for a total of 72. This is not so inadequate as it might seem, because only combinations that lead to extensive intramolecular hydrogen bonding will have low energy values. From the experimental side, nine new crystal structures of various sucrose derivatives (AHINAG, AHINIO, NIVPIR, QAMFAL, SAQYUD, TIKDEW, TIKDIA, VACZUV and VAGRUQ have been reported (see Table 1). Some fall into formerly unpopulated low-energy regions, vindicating our predictions.

METHODS

The calculations were carried out at the HF/6-31G* level of theory with the GAMESS computer program for the THF-O-THP and Me-THF-O-THP analogs (French et al., 2001). The MM calculations shown here were carried out with MM3(96), using the hydrogen bonding parameters from the 1992 version and a dielectric constant of 3.5, or with MM4. Several versions of MM4 have been tried during its development for carbohydrates in collaboration with Professor N. L. Allinger at the University of Georgia. Strategies for making the energy maps are detailed in French et al., 2000b. A total of 72 different combinations of exo-cyclic group orientations were included in these MM3 and MM4 calculations on sucrose at each ϕ, ψ point.

Experimental crystal structures were taken from the Cambridge Structural Database (CSD), with the latest updates as of January, 2004. Duplicate structures were excluded, as were those with additional covalent bonds connecting the fructosyl and pyranosyl rings. Twenty nine structures remain. They are identified by their CSD Refcodes, defined in Table 1. In three cases (HAHYUK, HEHXOG, ZZZNBS02), there were two different molecular shapes in the same crystal.

The distance in ϕ, ψ space from the sucrose crystal structure (SUCROS01) to any other structure is calculated by

$$d(\phi, \psi) = \sqrt{(\phi_{SUCROS01} - \phi_{OTHER})^2 + (\psi_{SUCROS01} - \psi_{OTHER})^2}$$

Table 1. Reference codes for the structures and citations.

REFCODE	Structure and Citation
AHINAG*	4,1',6'-Trichloro-4,1',6'-trideoxysucrose monohydrate. A. Linden, A. S. Muhammad Sofian, C. K. Lee (2002) <i>Acta Crystallogr., Sect. C: Cryst. Struct. Commun.</i> , 58 , o711
AHINIO*	2,3,3',6-Tetra-O-acetyl-4,1',6'-trichloro-4,1',4',6'-tetra(deoxygalactosucrose. A. Linden, A. S. Muhammad Sofian, C.K.Lee (2002) <i>Acta Crystallogr., Sect. C: Cryst. Struct. Commun.</i> , 58 , o718
CELGIJ	6-Kestose monohydrate. V. Ferretti, V. Bertolasi, G. Gilli, C. A. Accorsi (1984) <i>Acta Crystallogr., Sect. C: Cryst. Struct. Commun.</i> , 40 , 531
DINYOO10	O- α -D-Glucopyranosyl-(1-2)-O- β -D-fructofuranosyl-(6-2)- β -D-fructofuranoside
HAHXUJ	Sucrose sodium bromide dihydrate. C. A. Accorsi, F. Bellucci, V. Bertolasi, V. Ferretti, G. Gilli (1989) <i>Carbohydr. Res.</i> , 191 , 105
	Erlose monohydrate. T. Taga, E. Inagaki, Y. Fujimori, S. Nakamura (1993) <i>Carbohydr. Res.</i> , 240 , 39. O- β -D-Fructofuranosyl-(1-2)-O- α -D-glucopyranosyl-(1-4)- α -D-glucopyranoside monohydrate

HAHYUK	Xylosucrose. T.Taga, E.Inagaki, Y.Fujimori, K.Fujita, K.Hara (1993) <i>Carbohydr.Res.</i> , 241 , 63. β -D-Fructofuranosyl α -D-xylopyranoside hemihydrate.
HEGXOG	Erlose trihydrate. T. Taga, E. Inagaki, Y. Fujimori, S. Nakamura (1994) <i>Carbohydr. Res.</i> , 251 , 203. β -D-Fructofuranosyl 4-(O- α -D-glucopyranosyl)-D-glucopyranoside trihydrate
KANJOY	Sucralose. J. A. Kanters, R. L. Scherrenberg, B. R. Leeflang, J. Kroon, M. Mathlouthi (1988) <i>Carbohydr. Res.</i> , 180 , 175. 4,1',6'-Trichloro-4,1',6'-trideoxy-galacto-sucrose
KESTOS	1-Kestose. G.A.Jeffrey, Y.J.Park (1972) <i>Acta Crystallogr., Sect. B: Struct. Crystallogr. Cryst. Chem.</i> , 28 , 257
KSCOSF	Potassium sucrose-octasulfate heptahydrate. Y.Nawata, K.Ochi, M.Shiba, K.Morita, Y.Iitaka (1981) <i>Acta Crystallogr., Sect. B: Struct. Crystallogr. Cryst. Chem.</i> , 37 , 246
MELEZT01	Melezitose monohydrate. D. Avenel, A. Neuman, H. Gillier-Pandraud (1976) <i>Acta Crystallogr., Sect. B: Struct. Crystallogr. Cryst. Chem.</i> , 32 , 2598. O- α -D-Glucopyranosyl-(1-3)- β -D-fructofuranosyl- α -D-glucopyranoside monohydrate
MELEZT02	Melezitose monohydrate J.Becquart, A.Neuman, H.Gillier-Pandraud (1982) <i>Carbohydr.Res.</i> , 111 , 9. O- α -D-Glucopyranosyl-(1-3)- β -D-fructofuranosyl- α -D-glucopyranoside monohydrate
NEHCAE	Sucrose sarcosine monohydrate. R.V.Krishnakumar, S.Natarajan (1996) <i>Carbohydr.Res.</i> , 287 , 117. β -D-Fructofuranosyl- α -D-glucopyranoside N-methylglycine monohydrate
NIVPIR	6,6'-Dichloro-6,6'-dideoxy-2,3,4,3',4'-penta-O-benzylsucrose. Z.Ciunik, S.Jarosz (1997) <i>Pol. J. Chem.</i> , 71 , 207
PEKHES01	Nystose trihydrate. G. A. Jeffrey, De-bin Huang (1993) <i>Carbohydr. Res.</i> , 247 , 37. O- β -D-Fructofuranosyl-(2-1)-O- β -D-fructofuranosyl-(2-1)- β -D-fructofuranosyl α -D-glucopyranoside trihydrate
PELWEI	(6,6'-Diamino-6,6'-dideoxy-1',2,3,3',4,4'-hexa-O-methylsucrose-N,N')diiodo-platinum monohydrate acetone solvate. N. D. Sachinvala, Hong Chen, W. P. Niemczura, E. Furusawa, R. E. Cramer, J. J. Rupp, I. Ganjian (1993) <i>J. Med. Chem.</i> , 36 , 1791
PLANTE10	Planteose dihydrate. D. C. Rohrer (1972) <i>Acta Crystallogr., Sect. B: Struct. Crystallogr. Cryst. Chem.</i> , 28 , 425. α -D-Galactopyranosyl-(1,6)- β -D-fructofuranosyl-(2,1)- α -D-glucopyranoside dehydrate
QAMFAL*	2,3,6-Tri-O-acetyl-4-chloro-4-deoxy- α -D-galactopyranosyl 3-O-acetyl-1,4,6-trichloro-1,4,6-trideoxy- β -D-tagatofuranoside. C. K. Lee, H. C. Kang, A. Linden (1999) <i>J. Carbohydr. Chem.</i> , 18 , 241
RAFINO01	Raffinose pentahydrate. G. A. Jeffrey, De-bin Huang (1990) <i>Carbohydr.Res.</i> , 206 , 173. O- α -D-Galactopyranosyl-O- α -D-glucopyranosyl- β -D-fructofuranose pentahydrate
SAQYUD*	3-O-Acetyl-4-deoxy-4-iodo- β -D-fructofuranosyl 2,3,6-tri-O-acetyl-4-chloro-4-deoxy- α -D-glucopyranoside. A. Linden, C. K. Lee, A. S. M. Sofian (2001) <i>Acta Crystallogr., Sect. C: Cryst. Struct. Commun.</i> , 57 , 1098
STACHY01	Stachyose tetrahydrate. G. A. Jeffery, De-Bin Huang (1991) <i>Carbohydr. Res.</i> , 210 , 89 O- α -D-Galactopyranosyl-(1-6)-O- α -D-galactopyranosyl-(1-6)- α -D-Glucopyranosyl- β -D-fructofuranoside tetrahydrate
STACHY10*	Stachyose pentahydrate. R. Gilardi, J. L. Flippen-Anderson (1987) <i>Acta Crystallogr., Sect. C: Cryst. Struct. Commun.</i> , 43 , 806 O- α -D-Galactopyranosyl-(1-6)-O- α -D-galactopyranosyl-(1-6)-O- α -D-glucopyranosyl-(1-2)- β -D-fructofuranoside pentahydrate
SUCROS01	Sucrose. M. Bolte, M. Amon (2001) Private Communication. See also G. M. Brown, H. A. Levy. (1973) <i>Acta Crystallogr. Sect. B. Struct. Crystallogr. Cryst. Chem.</i> 29 , 790 or J. C. Hanson, L. C. Sieker, L. H. Jensen (1973) <i>Acta Crystallogr., Sect.B: Struct. Crystallogr. Cryst. Chem.</i> , 29 , 797
TIKDEW*	4-Bromo-4-deoxysucrose. A. Linden, C. Kuan Lee, A. S. M. Sofian (2001) <i>Acta Crystallogr., Sect. C: Cryst. Struct. Commun.</i> , 57 , 1363
TIKDIA*	1',6'-dibromo-4-fluoro-4,1',6'-trideoxysucrose monohydrate. A. Linden, C. Kuan Lee, A. S. M. Sofian (2001) <i>Acta Crystallogr., Sect. C: Cryst. Struct. Commun.</i> , 57 , 1363
VACZUV*	2,3,4,3'-Tetra-O-acetyl-4'-O-mesyl-6,1',6'-tri-O-tritylsucrose. A. S. M. Sofian, Cheang Kuan Lee, A. Linden (2002) <i>Carbohydr.Res.</i> , 337 , 2377
VAGRUQ*	(μ 2- β -D-Fructofuranosyl-O1,O3- α -D-glucopyranosid-O3',O4'-ato)-bis (ethylenediamine)-di-palladium(ii) undecahydrate. R. Ahlrichs, M. Ballauff, K. Eichkorn, O. Hanemann, G. Kettenbach, P. Klufers (1998) <i>Chem.-Eur.J.</i> , 4 , 835
ZZZNBS02	Tri-sodium bis(sucrose) tris(iodide) trihydrate. C. A. Accorsi, V. Bertolasi, V. Ferretti, G. Gilli (1989) <i>Carbohydr.Res.</i> , 191 , 91
ZZZSTI01	Sucrose octa-acetate. J.D.Oliver, L.C.Strickland (1984) <i>Acta Crystallogr., Sect. C: Cryst. Struct. Commun.</i> , 40 , 820

RESULTS AND DISCUSSION

Although Bock and Lemieux (1982) described the inter-residue linkage in sucrose as exceptionally rigid, the accumulated crystallographic data in Table 2 shows it to be very flexible for a carbohydrate. The change in this description is due to the much larger number of available crystal structures. Their conclusion was also due to the modeling method that they used. That

method used the atomic coordinates from the crystal structure of sucrose and they kept the internal geometries of the sugar rings fixed, unlike the work herein. Their rigid-geometry method leads to considerably higher relative energy values when the molecule takes values of ϕ and ψ different from those of the initial form, making the molecule appear to be more rigid. Based on the crystal structure data, the ϕ torsion angle ranges over more than 60° . ψ has two ranges, one of 90° , plus a single value for KANJOY (sucralose), 118° away from crystalline sucrose. This flexibility is illustrated by superposition of the different molecules, as shown in Figure 3.

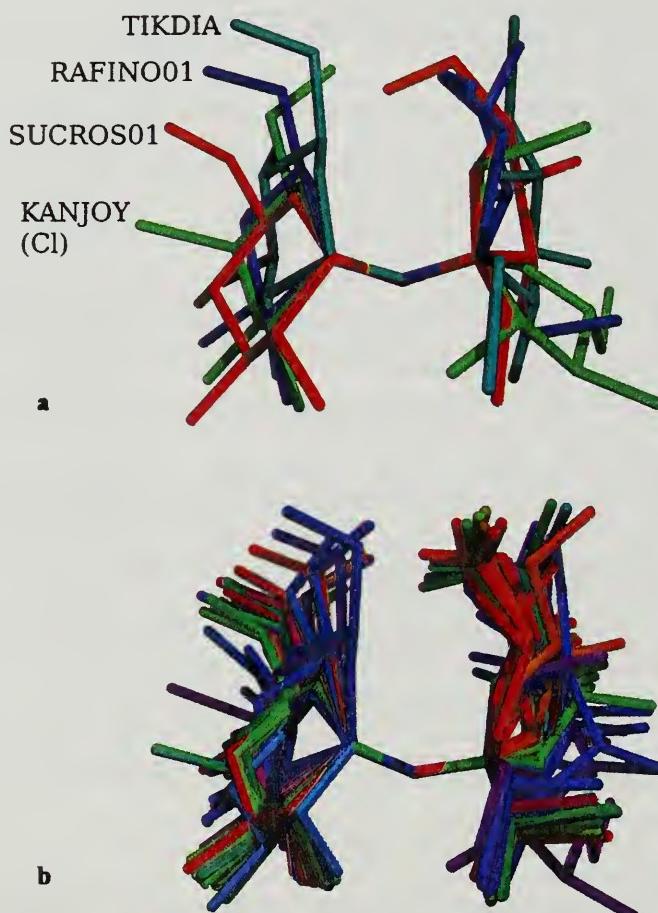


Figure 3. Superposition of atoms C1, O1, and C2' with glucopyranosyl rings on the left and fructofuranosyl rings on the right. a. 4 selected sucrosyl moieties from experimental crystal structures, labeled with their REFCODES. b. 29 sucrosyl moieties from experimental crystal structures.

Table 2 also shows the values of the primary alcohol torsion angles, as well as a characterization of the fructofuranosyl ring. There are four structures with the “Southern” E₄ form of the ring. The C6–O6 groups on the glucosyl ring take values near $+60^\circ$ or -60° and do not participate in inter-residue hydrogen bonds. The C1'–O1' groups take values near -60° or 180° , with all inter-residue hydrogen bonds corresponding to values near 180° . The C6'–O6' groups with values near -60° all make hydrogen bonds to O5. With one weak exception (HEGXOG), values near $+60^\circ$ and 180° do not result in inter-ring hydrogen bonds. In the main ϕ , ψ region, inter-ring hydrogen bonds occur only in a sub-part, with $95^\circ < \phi < 122^\circ$; $-50^\circ < \psi < -30^\circ$. That same sub-part is inhabited by other structures that do not form hydrogen bonds.

Table 2. Linkage and primary alcohol torsion angles and furanosyl ring shapes for crystalline sucrose moieties

Refcode	$\phi_{o5c1o1c2'}$	$\psi_{o5'c2'o1c1}$	$\chi_{o5c5c6o6}$	$\omega_{o5'c2'c1'o1'}$	$\chi'_{o5'c5'c6'o6'}$	ring shape
AHINAG*	61.02	-78.80	-72.9	60.0 (F)	58.2(F)	4T_3
AHINIO*	113.88	-46.06	65.6 (S)	-166.6(Cl)	71.7(Cl)	4E
CELGIJ	89.58	-54.50	63.2	-63.8	67.9 (S)	4T_3
DINYOO10	99.84	-46.06	-62.4	-53.1	-74.9	4E
HAHXUJ	104.54	-32.56	73.8	-61.3	64.0	4T_3
HAHYUK	96.95	-37.18	NA	-56.6	-63.3 hb O5 , 66.9	4T_3
HAHYUK	108.25	-46.90	NA	172.8 hb O2	61.4	E_3
HEGXOG	109.9	-39.76	-64.5	-51.0	62.5	E_3
HEGXOG	98.00	-55.71	-71.1	-58.3	50.3 hb O5 wk	E_3
KANJOY	91.43	-162.21	66.9	-59.2 (Cl)	61.3 (Cl)	4E O2-O3'hb
KESTOS	84.64	-65.90	NA	179.2 (S)	64.6	E_4
KSCOSF	107.25	-41.52	-62.3	-65.3	-177.1	E_4
MELEZT01	99.77	-30.61	-64.6	-62.7	-65.0 hb O5	4E
MELEZT02	109.58	-43.42	-48.3	-63.7	57.3	E_3
NEHCAE	93.96	-20.58	-53.1	-54.8	70.0	4T_3
NIVPIR	105.79	-52.96	-65.3 (Cl)	173.8 hb O2	169.1 (Cl)	4E
PEKHES01	102.33	-18.64	-60.3	-63.3 (S)	68.9	4T_3
PELWEI	110.32	-70.29	48.4 (N)	-73.5 (S)	63.4 (N)	4E
PLANTE10	108.21	-26.18	63.5	-65.6	63.5 (S)	E_3
QAMFAL*	113.07	-47.97	65.0 (S)	-63.6 (Cl)	70.8 (Cl)	4E
RAFINO01	82.08	11.98	-63.1 (S)	-60.1	68.0	4T_3
SAQYUD*	104.75	-42.47	-71.8 (S)	-58.3	-64.4 hb O5	4E
STACHY01	109.32	-47.31	-62.0 (S)	-178.4 hb O2	-63.3 hb O5	Disorder
STACHY10*	108.79	-47.91	-61.6 (S)	-178.6 hb O2	-63.5 hb O5	4T_3
SUCROS01	108.34	-44.67	-56.5	171.1 hb O2	-70.1 hb O5	E_3
TIKDEW*	107.31	-32.23	-65.7	166.6 hb O2	74.0	E_3
TIKDIA*	61.53	-78.92	-71.7	-177 (Br)	60.1 (Br)	4T_3
VACZUV*	70.39	-81.99	-57.6 (S)	-179.1 (S)	-167.1 (S)	E_4

Refcode	$\phi_{O5-C1-O1-C2'}$	$\psi_{O5'-C2'-O1-C1}$	$\chi_{O5-C5-C6-O6}$	$\omega_{O5'-C2'-C1'-O1'}$	$\chi'_{O5'-C5'-C6'-O6'}$	ring shape
VAGRQU*	122.38	-50.50	-55.0	173.0(C) O2 O1'	65.3	E_3
ZZZNBS02	79.56	-61.60	56.9	-177.5	53.8	E_5
ZZZNBS02	79.93	-66.53	61.3	172.6	55.8	4E
ZZZSTI01	93.22	-21.79	65.5 (S)	-59.8 (S)	178.5	E_4

Figures 4-6 show, respectively, energy surfaces based on the Me-THF-O-THP analog calculated with HF/6-31G* quantum mechanics, sucrose calculated with the hybrid method, and sucrose calculated with MM4. All of the methods indicate that most crystal structures are in the lowest energy regions, supporting the flexibility deduced from the crystal structures. The unpopulated local minimum at $\phi = 85^\circ$, $\psi = 50^\circ$ is the only obvious problem for the Me-THF-O-THP surface. Figure 5 is similar to our earlier hybrid map. Figure 6 was made with a modified version of MM4, development of which is continuing. It shows that a purely empirical method can predict sucrose structures, but there are other problems with these modifications.

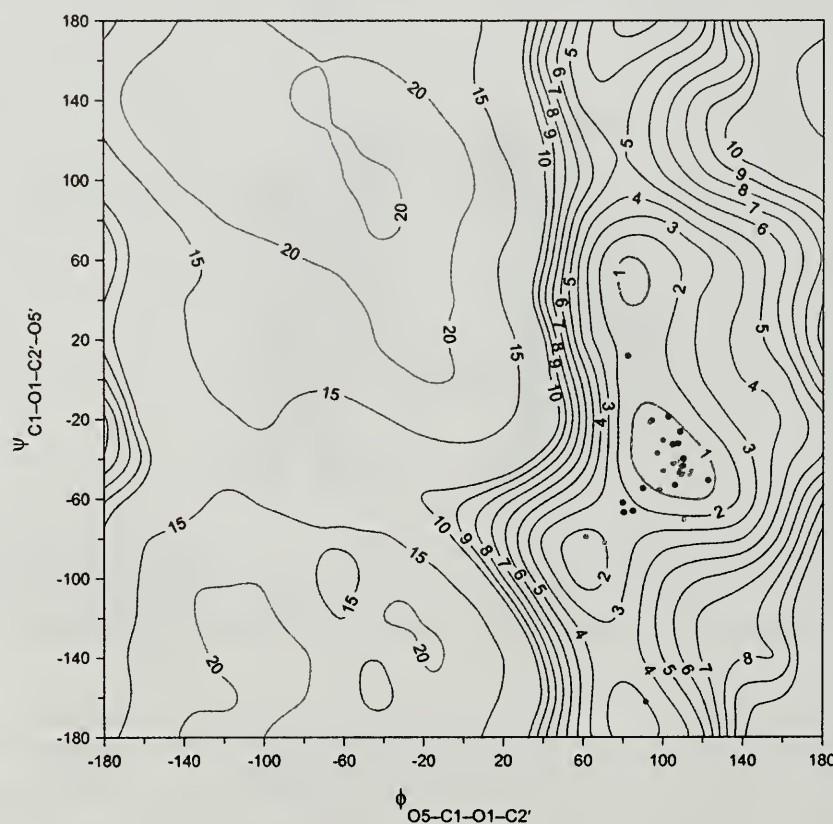


Figure 4. THP-O-1-Me-THF Sucrose analog. HF/6-31G* calculations, and linkage conformations that are observed in crystal structures are shown as colored dots that indicate intramolecular hydrogen bonding, if any. The color key is in Figure 7, except for the green dot at $\phi = 91^\circ$, $\psi = -162^\circ$ (KANJOY).

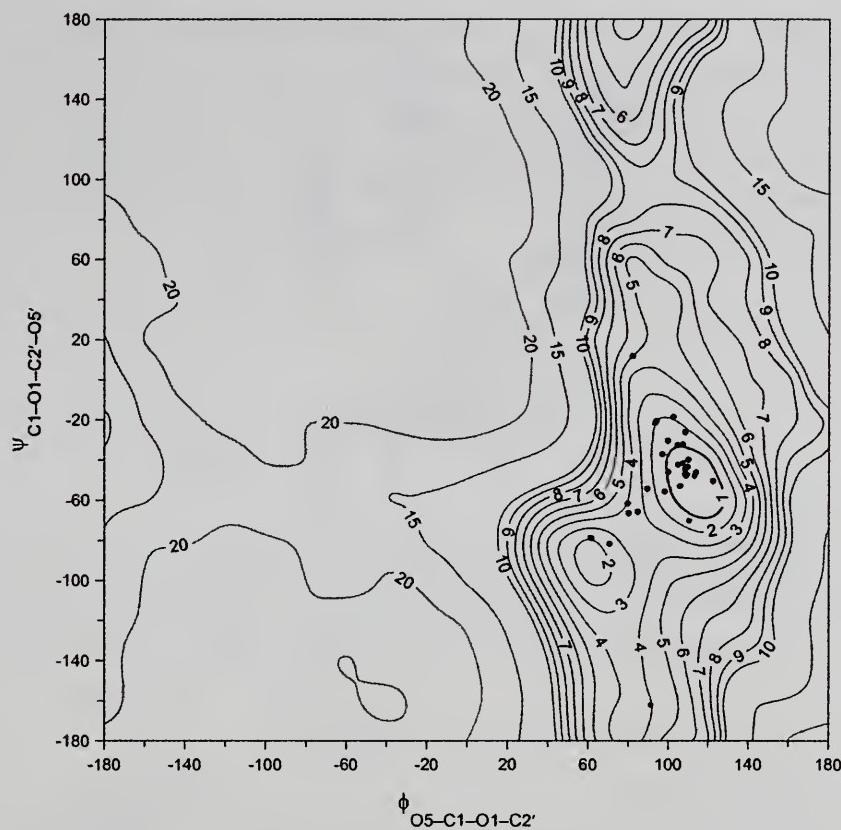


Figure 5. Sucrose. HF/6-31G*::MM3 hybrid. See Figure 4 for details.

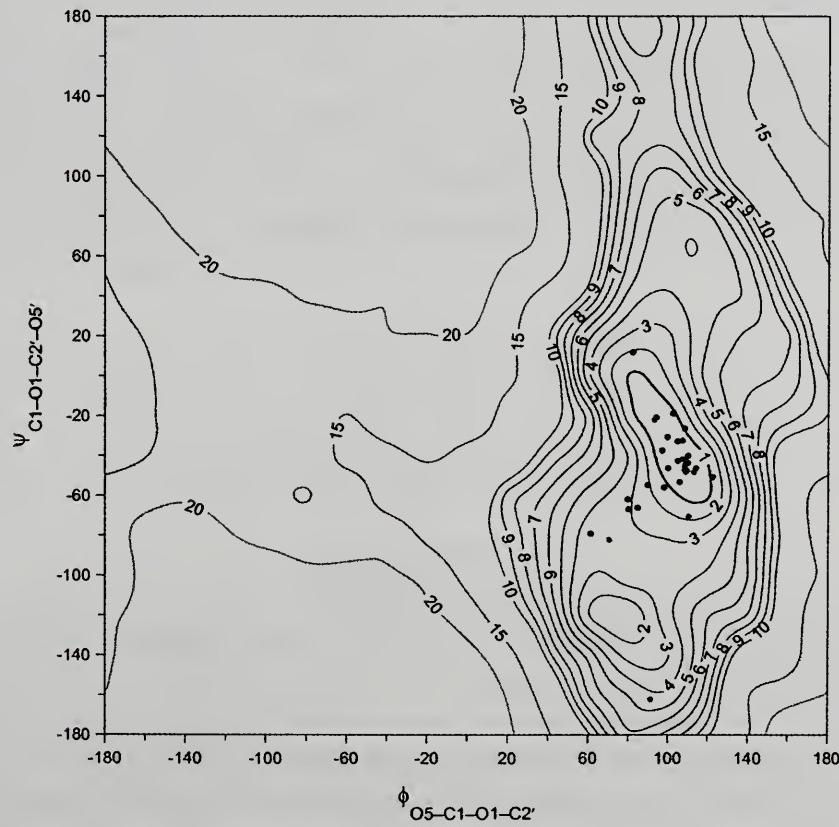


Figure 6. Sucrose. MM4, with custom torsion parameters. See Figure 4 for details.

Figure 7 is a zoomed-in view of the ϕ , ψ map that identifies the different crystal structures, except KANJOY. The 1-kcal/mol energy contours from Figures 4-6 are shown, as are the extents of hydrogen bonding for each structure. Only the SUCROS01 and STACHY structures have two inter-residue hydrogen bonds, and they are separated by only $d(\phi, \psi) = 3^\circ$. Structures having only a hydrogen bond between O1' and O2 have $\phi > 105^\circ$, while structures having a single hydrogen bond between O6' and O5 have $\phi < 105^\circ$.

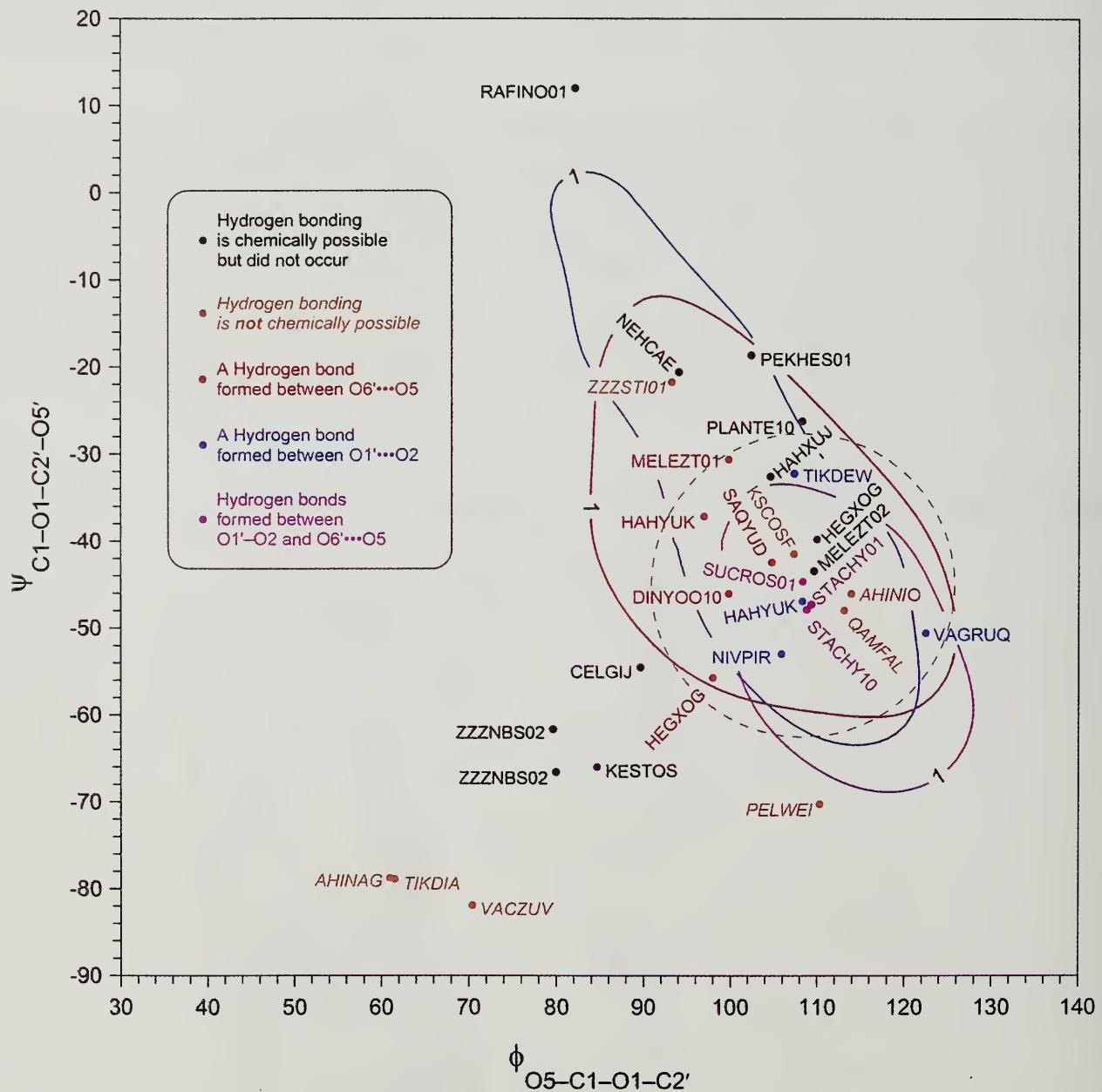


Figure 7. The grey dashed circled is centered on SUCROS01 and encompasses all of the crystal structures with at least one hydrogen bond.

Figure 8 shows a superposition of SUCROS01 and MELEZT02, which are only 1.8° apart in ϕ , ψ space. In SUCROS01, both hydrogen bonds are formed, while neither are formed in MELEZT02, despite the obvious opportunity to do so. Instead, the O1' and O6' hydroxyls are directed at other molecules. KSCOSF is only 3° distant in ϕ , ψ space from SUCROS01 despite being completely sulfated and unable to form any inter-residue hydrogen bonds.

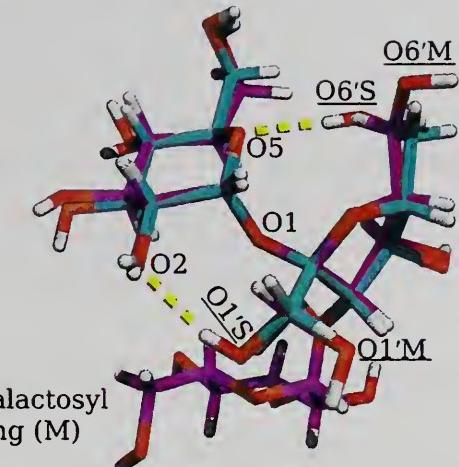


Figure 8. Superposition of SUCROS01 (S, blue/green) and MELEZT02 (M, magenta) structures based on C1, O1, and C2'. Hydrogen bonds in SUCROS01 are shown as dashed lines. Underlines designate rotated primary alcohols on the fructose ring.

CONCLUSIONS

The flexibility of sucrose is indicated by a wide range of experimentally observed shapes. The limited influence of intramolecular hydrogen bonds is shown by the large number of sucrose-type structures that do not make such bonds. The preferred shapes are indicated by a high density of observed shapes within a substantially restricted range. The fact that the linkage geometries of sucrose, melezitose and sucrose octasulfate are nearly identical shows that these shapes are preferred regardless of formation or even ability to form hydrogen bonds. We infer that hydrogen bonds can, but do not always, form when the shape of the molecule permits them. Agreement between the Me-THF-O-THP quantum mechanics map and the crystal structures means that the forces that determine the shape of isolated models of Me-THF-O-THP also determine the shape of sucrose in crystalline phases. The same dependence on those forces may apply to the shape of the sucrose molecule in aqueous solution.

Problems in predicting sucrose with standard MM software indicate the difficulty in obtaining consistent empirical functions that reproduce the complex stereochemistry of the glycosidic linkage in sucrose. If crystals of sucrose derivatives not in Table 1 are found, it would be very useful to have their structures determined. Each one is an important piece of the big picture.

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A RAPID STARCH TEST FOR USE IN CANE MILLS

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ABSTRACT

Starch is an important quality factor in raw sugar production and sugar refining. Starch concentration is higher in cane juice from immature cane plants and in green (not burned) cane, now harvested more frequently in all parts of the world. There is a need for a rapid and simple method that can be used in cane mills. A simple, rapid, and quantitative test for starch in cane juice and raw sugar has been developed by Sugar Processing Research Institute (SPRI) for use in cane mills. The method showed good correlation (>95%) with the standard method. It can be used on raw juice, clarified juice and raw sugar. The method can be easily learned, uses equipment already on hand in most mill laboratories, and can be integrated into current laboratory practices. The method was successfully tested in a Louisiana mill over a grinding season and also on cane juice representing numerous sources and a wide concentration range of starch. This paper also reviews the behavior of starch in the mill during the grinding season.

INTRODUCTION

Starch is an important quality factor in raw sugar production as well as in sugar refining. It arises in the actively growing part of the sugarcane plant (apical meristem and leaves) and is higher in immature cane. Varieties can differ widely in the amount of starch in juice, from 200 ppm on Brix (CP 70-312) to >1200 ppm (LCP 85-384). Starch in process streams at the mill increases viscosity, slows crystallization and purging, increases molasses purity and is carried into raw sugar. Since starch tends to be high in Louisiana, most mills dose amylase enzyme continuously. High starch (250 ppm and above) negatively affects refinery operations.

In recent years, the change to green cane harvesting (cane that is not burned) and harvesting of immature cane, along with new higher starch varieties, have all contributed to a higher quantity of starch in cane juice.

Most starch tests are based on alcohol precipitation of starch from sugar solutions and measure the intensity of the blue color produced by the starch-iodine complex (Balch, 1953a, b; Matic, 1971; Godshall, 1990). Refineries and mills alike have adopted a variety of modifications of these methods, with the result being that there is seldom agreement among laboratories on the specific starch content of the same sample (Matic, 1971). Mill and refinery quality control managers have also reported adopting a method of testing process streams directly using an iodine solution (Personal Contact a, 1999 and b&c, 2000) or adding iodine reagent directly to an acidified sample (Charles, 1968). These methods are largely qualitative.

SPRI Rapid Starch Method

The SPRI Rapid Starch Test is based on measuring absorbance of the blue starch-iodine complex. In the test, starch in cane juice or raw sugar is first gelatinized (solubilized) by boiling for 5 min. The solubilized starch is then reacted with iodine to form a blue/purple starch-iodine complex. The sample is briefly clarified by centrifugation. The absorbance of the reacted starch is read at 600 nm. The μg of starch in the sample is determined from the starch calibration curve. When the reagent is added, a blue/purple color momentarily forms, then is masked by the orange color produced by the formation of excess iodine. The over-all color of the reacted solution will be orange-brown. The spectrophotometric reading at 600 nm will read only the blue color, so the orange color of the iodine will not interfere. No alcohol precipitation step is needed. The test takes about 15 minutes, and four to six samples can be run simultaneously. The equipment required is routinely available in cane mills, and consists of a table top centrifuge with a speed of about 3000 rpm, a hot plate, a spectrophotometer and an analytical balance.

The SPRI Rapid Starch Test method is shown in Appendix 1.

The method for establishing the starch calibration curve is shown in Appendix 2.

MATERIALS AND METHODS

The method development work was done at the SPRI laboratories in New Orleans, but it was desirable to work on site to make sure that the method was indeed robust and possible to conduct routinely in a mill laboratory setting. SPRI personnel traveled to a mill in Louisiana, taking all the equipment needed, including a spectrophotometer, tabletop centrifuge, all glassware and reagents on 5 occasions during the 2001 grinding season: October 18, November 1, November 15, November 29 and December 9. As we gained experience with the pitfalls and problems of on-site testing, the method was modified as required. For example, we originally used glass centrifuge tubes, but one broke in the centrifuge, so the method was changed to use plastic tubes. We also found it necessary to increase the boiling time of raw mixed juice from 3 minutes to 5 minutes. We experimented with microwave heating, but found that boiling in a hot water bath gave uniformly consistent results, whereas microwave heating was inconsistent and unsatisfactory, sometimes leading to splattering and loss of sample.

In the mill, duplicate samples of raw mixed juice and clarified juice were obtained at 9:00 am, 10:00 am and 11:00 am on each testing occasion. An appropriate aliquot of each sample of raw juice and

clarified juice was precipitated in alcohol for determination of starch by the standard SPRI method later at the SPRI labs. A composite sample of raw sugar was obtained on each occasion and analyzed later by both the standard and the rapid methods.

During development and validation of the method, many other samples of raw sugar and juice were analyzed at the SPRI labs. Results of the rapid test were compared to the standard SPRI method. The standard method is the SPRI adaptation of the SMRI method, and is described in Godshall, et al (1990). In that test, starch is precipitated by 80% aqueous ethanol, the precipitated starch trapped on a mat of filter aid and solubilized by boiling in a calcium chloride solution. The isolated starch is reacted with iodine and colorimetrically analyzed as the blue starch-iodine complex at 600 nm.

RESULTS AND DISCUSSION

Boiling Time

It was necessary to increase the boiling time for raw juice from 3 minutes (the original time stated in the method) to 5 minutes, since all of the starch was not solubilized in 3 minutes, and the results were too low when compared to the standard test. Most of the starch in the juice was solubilized by the high heat of clarification in the mill, so a 3 minute heating time for clarified juice was sufficient. Raw sugar generally did not need to be boiled. Figure 1 compares the results of starch in raw sugar by the rapid method with heated and unheated raw sugar samples. The results showed that within the range of starch concentration normally seen in Louisiana raw sugars, there was no significant difference between boiling and not boiling the sample prior to reacting with the reagents. However, for the sake of consistency, all samples, including raw sugar, are to be boiled for 5 minutes.

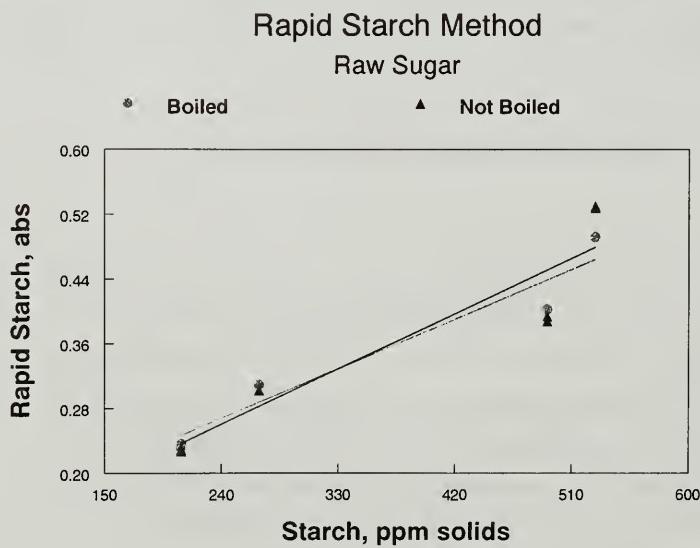


Figure 1. Comparison of results by the rapid starch method for raw sugar, boiling vs. not boiling the sample. The results show that within the range of starch concentration normally seen in raw sugars, there was no significant difference between boiling and not boiling the sample.

Type of Starch to Use for the Calibration Curve

The type of starch used for preparing the calibration curve is very important. It must be soluble, consistent and commercially available. Potato starch fulfills these requirements. Questions are often asked as to why not use cornstarch, which is assumed to be more like cane starch, given the botanical affinity of the two plants.

Commercial potato starch, rice starch, wheat starch, and cornstarch were all used to develop standard starch calibration curves. Figure 2 compares the standardization curves developed with cornstarch, potato starch and rice starch. Wheat starch gave a curve similar to rice and corn, but is not shown.

Although rice, corn and wheat starch all produced excellent regression lines, they produced a lower slope than cornstarch because of less color development. Calibration curves with lower absorbance will yield higher starch values in test materials. In addition, corn, wheat and rice starch all produced hazy solutions, even when boiled for 5 minutes in calcium chloride solutions, indicating either starch insolubility, or, more likely, the presence of insoluble material, such as protein, lipid or granule debris. For this reason, potato starch from Sigma is recommended as the standard starch.

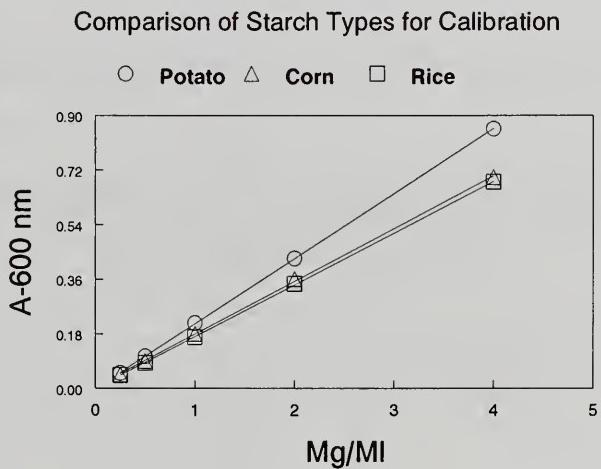


Figure 2. Calibration curves developed with potato starch, cornstarch and rice starch.

Comparison of the Standard Test with the Rapid Test

Figure 3 shows the results of comparing 52 samples of raw and clarified juices over a wide range of concentrations (250 ppm to 3500 ppm). The correlation was close to 0.99 and the correspondence was close to one-to-one.

Figure 4 shows the results of comparing 24 raw sugars over a wide range of concentrations (80 ppm to 600 ppm), which represents the typical ranges found in Louisiana raw sugars. The correlation was 0.98, and the correspondence, again, was close to one-to-one. The data for this graph are shown in Table 1.

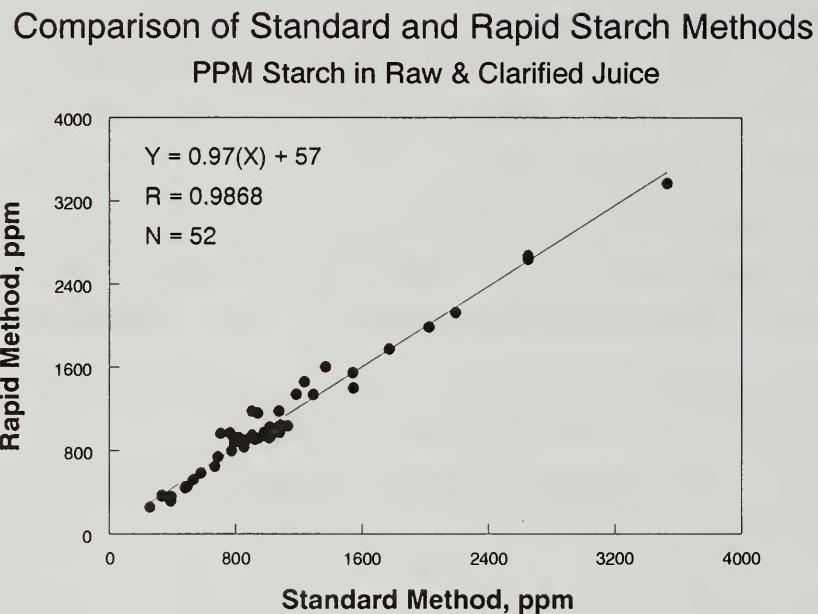


Figure 3. Comparison of rapid method with standard method for raw and clarified cane juice.

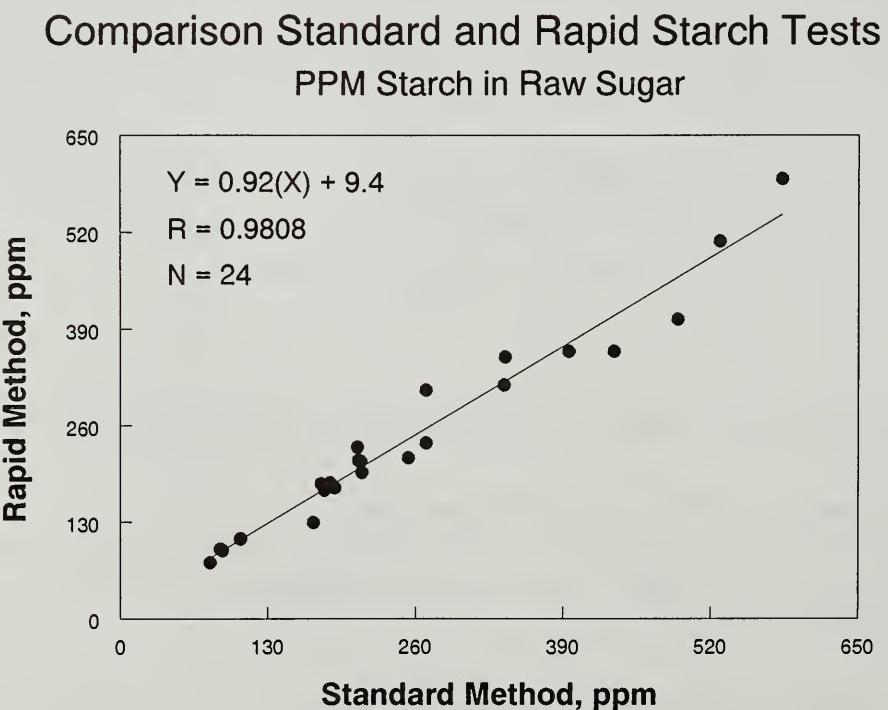


Figure 4. Comparison of rapid method with standard method for raw sugar.

Table 1. Starch in raw sugar - comparison of SPRI standard starch method and SPRI rapid starch method.

Sample	Standard method	Rapid method
1	79	76
2	88	94
3	90	92
4	106	108
5	106	107
6	170	130
7	177	182
8	180	173
9	185	183
10	189	176
11	209	231
12	210	213
13	212	212
14	213	197
15	254	217
16	269	237
17	269	308
18	338	315
19	339	353
20	395	360
21	435	360
22	491	403
23	528	508
24	583	592

Does Native Amylase in the Mixed Juice Degrade Starch over Time?

We also wished to determine if a delay of analysis might lead to lower starch results caused by degradation of the starch by the native amylase enzyme in the mixed juice. A sample of mixed juice was obtained and left to sit on the lab bench, with aliquots taken for analysis at 0, 15, 30, 45, 60 and 120 min. Figure 5 shows the results for four dates.

The data showed that there may have been a small decrease over time, but it was not significant and was not consistent. The largest decrease was shown on Nov. 15. The variation from the means of these daily results was not large enough to indicate a significant change in starch content over a 2-

hour period: Mean of 11/1/01 = 1370 ppm \pm 174; mean of 1/15/01 = 1025 ppm \pm 116; mean of 11/29/01 = 1521 \pm 67. The differences may be caused as much by uneven distribution of the insoluble starch granules in the juice (they will have a tendency to settle out). It is, of course, not recommended to leave mixed juice out for any length of time because of the rapid microbial degradation that can take place that will affect other analyses and quality parameters. Following clarification, native enzymes are, in all probability, inactivated by the high heat. The results indicated that natural amylase did not play a significant role in lowering the starch content in mixed juice on standing at room temperature for up to two hours.

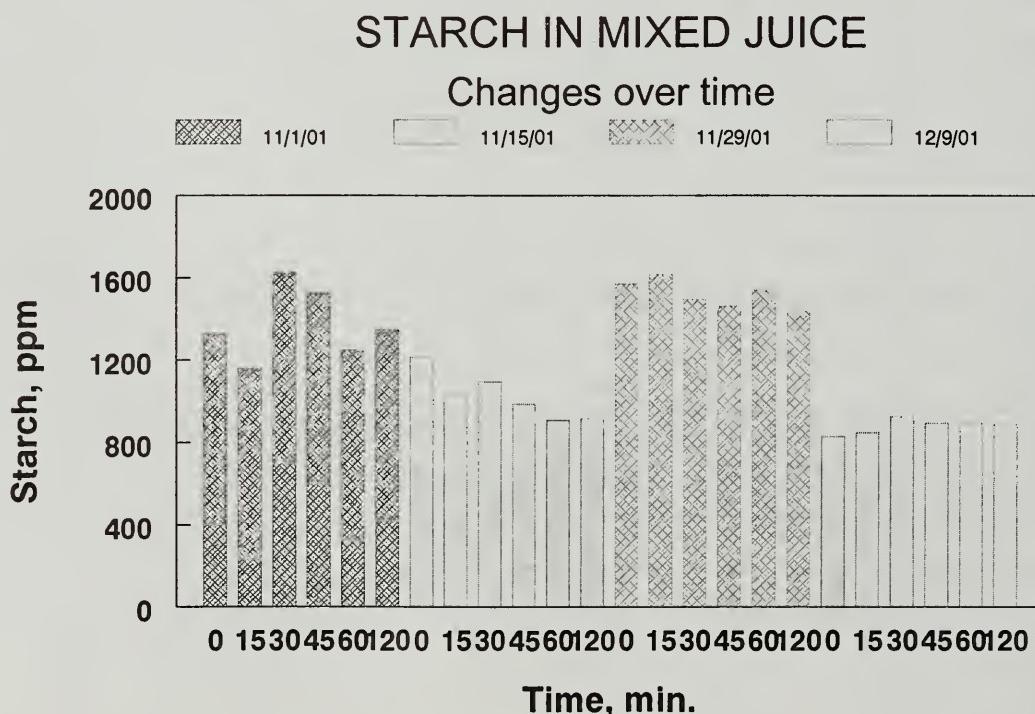


Figure 5. Change in starch content raw mixed juice after being allowed to stand from 0 minutes to 2 hours at room temperature.

Behavior of Starch in the Mill Over the Season

It was of interest to note if clarification removed starch. Figure 6 shows the difference between raw juice and clarified juice starch levels. There was little to no removal of starch during clarification. The major trend noted was the large drop in starch in juice over the season, as the cane continued to mature. The mixed juice represented cane from different farmers, different varieties (the majority was from cane variety LCP 85-384, a high starch cane that averages 1000-1200 ppm starch in raw juice), and possibly different field and cane handling conditions.

The mean starch in raw mixed juice over the period studied was 1054 ppm, and in clarified juice, it was 884 ppm. This would indicate approximately 16% reduction in starch by clarification. Given

the high levels of starch in the raw juice, this is not a significant decrease. If it is estimated that 30 to 60 percent of the starch in evaporator juice will carry into the raw sugar crystal on the first strike, and 250 ppm is an approximate cut-off point for refinery operations, there was a need for almost constant dosing of amylase enzyme during the season at this location.

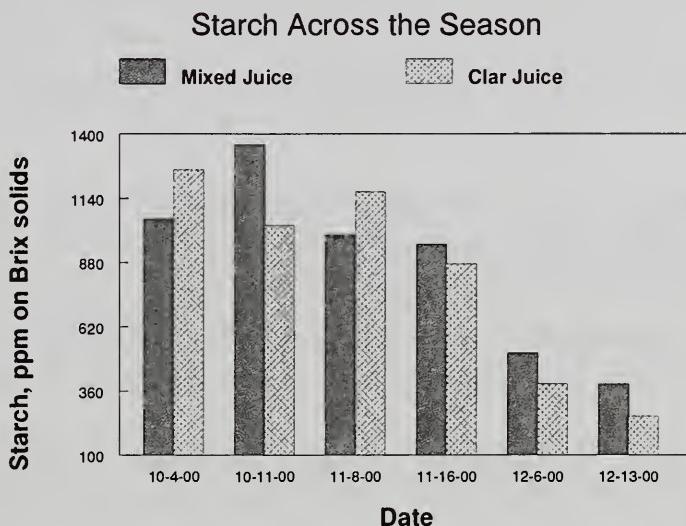


Figure 6. Changes in starch over clarification and over the harvest season.

CONCLUSION

A simple, rapid, quantitative starch method for mixed juice, clarified juice and raw sugar has been developed and validated. It takes 15-20 minutes to complete, and multiple samples can be run at the same time. Very small amounts of reagents are required and all equipment needed is already in common use in mill labs. The cost of reagent per sample is low, on the order of \$0.10-0.15.

This study has confirmed the high levels of starch in Louisiana cane juice. Although the data from this mill showed the necessity to dose starch continuously, as other cane varieties are harvested, and in other locations, this test can be used to determine when amylase enzyme needs to be dosed. It can also be adapted to measure starch in evaporator syrup to determine if the enzyme is functioning at a proper level.

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APPENDIX 1

SPRI RAPID STARCH TEST

Scope and Principle

Starch in cane juice and raw sugar is first gelatinized (solubilized) by boiling for 5 min. The starch is then reacted with iodine to form a blue/purple starch-iodine complex. The absorbance of the reacted starch is read at 600 nm. The μg of starch in the sample is determined from the starch calibration curve. The method is applicable to all process samples

Upon addition of the KIO_3 , a blue/purple color will momentarily form, then will be masked by the orange color produced by the formation of excess iodine. The over-all result will be an orange-brown color in the reacted sample. The spectrophotometric reading at 600 nm will read only the blue color, so the orange color of the iodine will not interfere.

Equipment

Hot plate

Boiling water bath (Alternatively, a large steel bowl and a metal test tube rack that fits inside)

Analytical balance

Spectrophotometer

Table top centrifuge, speed of about 3000 rpm.

Supplies

Conical plastic test tubes with caps, 15 ml capacity.

Beakers

Liter volumetric flasks (2)

10-ml volumetric flask (1)

Automatic pipetor, 0.25, 1.2, 2.5, and 3- ml capacity

1-cm cuvets for spectrophotometer (disposable plastic cuvets are adequate)

Reagents

Deionized water

Acetic acid, 2N

Potassium iodide (KI), 10%

Potassium iodate (KIO_3), M/600

Preparation of Reagents

2N acetic acid 120 g glacial acetic acid in one liter

10% KI 1 g KI /10 ml (prepare fresh daily)

KIO_3 , M/600 Dissolve 0.3567 g KIO_3 in 1 L (prepare fresh weekly)

Procedure for Raw and Clarified Juice

1. Determine the Brix of each sample to be analyzed
 2. Pipet 3 ml cane juice into a test tube
 3. Place in a boiling water bath for 5 min. Allow to cool to the touch, about 1-2 min.
 4. To each of the sample test tubes, pipet the following:
 1.2 ml 2 N acetic acid
 0.25 ml 10% KI
 2.5 ml KIO_3
 5. For the blank, pipet the following into one test tube:
 3 ml water
 1.2 ml 2 N acetic acid
 0.25 ml 10% KI
 2.5 ml KIO_3
 6. Mix the contents of the test tubes thoroughly by inverting several times.
 7. Centrifuge the test tubes for 5 min
 8. Blanking procedure: Zero the spectrophotometer using the blank sample
 9. Read the absorbance of the sample at 600 nm.
 10. Determine the μg of starch from the calibration curve.
 11. Calculate ppm starch on Brix solids.

To calculate the ppm starch in a juice sample

$A = \mu\text{g starch}$ (obtained from the calibration curve)

B = ml sample (3 ml)

C = g sucrose/ml in juice (from Brix tables, eg ICUMSA SPS-4)

$$\text{ppm starch} = \frac{A}{B \times C}$$

Example:

300 µg starch from the calibration standard curve; sample = 13.2 Bx.

From the tables, 13.2 Bx \Rightarrow 0.1388 g sucrose/ml

$$\text{ppm starch} = \frac{A}{B \times C} = \frac{300 \mu\text{g}}{(3 \text{ ml}) (0.1388 \text{ g/ml})} = 720 \mu\text{g starch/g sucrose} = 720 \text{ ppm}$$

Procedure for Determination of Starch in Raw Sugar by the Rapid Test

1. Prepare a 15 Bx solution of raw sugar: 15 g sugar + 85 g water
 2. Stir to dissolve thoroughly.
 3. Transfer 3 ml of the raw sugar solution into a test tube
 4. Follow the same procedure as for cane juice above, starting with step 3.

To calculate the ppm starch in a raw sugar

$A = \mu\text{g starch}$ (obtained from the calibration curve)

B = ml sample (3 ml)

C = g sucrose/ml in juice (from Brix tables; for 15 Bx = 0.1589)

$$\text{ppm starch} = \frac{A}{B \times C} = \frac{A}{(3)(0.1589)} = \frac{A}{0.4767}$$

Example:

75 µg starch from the calibration curve; sample = 15.0 Bx.

From the tables, $15.0 \text{ Bx} \Rightarrow 0.1589 \text{ g sucrose/ml}$ (this is a constant number)

$$\text{ppm starch} = \frac{A}{0.4767} = \frac{75 \mu\text{g}}{0.4767} = 157 \mu\text{g starch/g sucrose} = 157 \text{ ppm}$$

APPENDIX 2

CALIBRATION CURVE FOR RAPID STARCH TEST

Prepare a Stock Starch solution of soluble potato starch to contain 1 mg starch/ml. (An example of commercial soluble potato starch is Sigma #S-2004).

1. Determine the moisture content of the starch by drying 10 grams overnight in a 94-100° C oven. The difference in weight between the “wet” and dried starch divided by the original wet weight of starch times 100% is the moisture content:

$$\% \text{ moisture in starch} = \frac{(\text{wet wt} - \text{dry wt})}{\text{wet wt}} \times 100\%$$

Discard the dried starch after determining the moisture content, as it is retrograded and will not go into solution. For the calibration curve, use only unheated starch and adjust the weighed amount for the calculated moisture content. (See No. 2 below.)

2. Determine how much starch is needed to obtain the equivalent of 500 mg of dry starch: For example, if the moisture content was 10.5%, 500 mg dry starch = $500/(1.00 - 0.105) = 558.66$ mg starch is needed to give the equivalent of 500 mg dry starch.

3. Make a stock solution of starch to contain 1 mg starch/ml:

- a) 500 mg dry starch equivalent (calculated from No. 2 above) is slurried with about 10 ml water in a 100-ml beaker.
- b) This is quantitatively added to 300 ml boiling water in a 1 Liter beaker, and boiling is continued for 1 min.
- c) Allow the solution to cool.
- d) Transfer quantitatively to a 500 ml flask and adjust to volume with water.

4. Make up the following standard starch solutions: Into separate 100-ml flasks, add 2.5 ml, 5 ml, 10 ml, 20 ml, 30 ml of the starch stock solution, and adjust to volume with water. Mix well. These solutions contain, respectively, 0.025 mg starch/ml; 0.05 mg starch/ml; 0.10 mg starch/ml; 0.20 mg starch/ml; and 0.30 mg starch/ml.

5. Pipet 3 ml from each of the standard solutions and place in 15-ml conical test tubes with screw tops. Each test tube will contain, respectively, 75 µg, 150 µg, 300 µg, 600 µg, and 900 µg of starch.

6. For the blank, pipet 3 ml water into a 15-ml conical test tube.

7. To each of the test tubes containing standard starch solutions and the blank add the following reagents in order: (For preparation of reagents, see SPRI Rapid Starch Test Method.)

1.2 ml 2 N acetic acid

0.25 ml 10% KI

2.5 ml KIO_3

8. Mix contents well and read the absorbance at 600 nm.

9. Plot the curve with the μg starch on the X (bottom) axis and the absorbance on the Y axis.

Figure 1 shows typical calibration curves obtained using two common laboratory spectrophotometers, a Spectronic D and a Shimadzu.

Data

μg Starch	Shimadzu	Spectronic
0	0	0
75	0.160	0.158
150	0.334	0.332
300	0.651	0.648
600	1.283	1.300
900	1.853	1.950

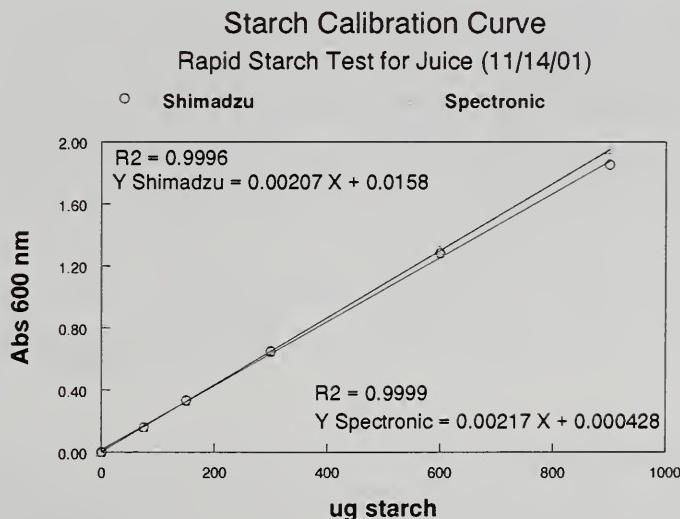


Figure 1. Calibration curves obtained with different spectrophotometers.

COLLABORATIVE STUDY ON STARCH IN RAW SUGAR USING THE SPRI RAPID STARCH METHOD

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ABSTRACT

Residual starch in cane sugar can be an important quality factor during refining. Many methods for starch analysis exist, but there is no standardized method. At its August 2003 meeting, the U.S. National Committee on Sugar Analysis recommended that SPRI organize a collaborative study within the United States on the recently developed SPRI rapid starch test. Fourteen laboratories participated, representing refineries, raw sugar mills and laboratories throughout the United States. Six raw sugars, with starch concentrations ranging from 112 to 746 ppm were analyzed as blind duplicates. The results indicated that the variability of the method fell within the parameters of an acceptable collaborative study, with the Horwitz ratio (a criterion used to assess reproducibility) falling below 2.0, except for one sample. Since thirteen of the fourteen participating labs were unfamiliar with the method, this was an excellent outcome, and it is recommended that this method be adopted for determining starch in raw sugar.

INTRODUCTION

A rapid starch test was developed by Sugar Processing Research Institute, Inc., which could be used for clarified cane juice, syrup and raw sugar. (Refer to paper by Godshall, et al, this volume, for details of the method.) The method was intended for use in cane mills, and designed to use readily available and economical equipment already present in most mill laboratories. At its August, 2003, meeting, the members of the U.S. National Committee on Sugar Analysis recommended that the method be submitted to a collaborative test within the U.S.

Fifteen laboratories in the United States indicated an interest in participating. Of these, 14 labs returned results. These labs represented a good cross section of the U.S. cane sugar industry, including refineries, laboratories and mills in the three cane producing states, Florida, Louisiana and Texas. The participating laboratories are listed in Table 1. The list is in alphabetical order and does not correspond to the assigned lab numbers in the rest of the report. None of the labs, except for SPRI, were familiar with the method.

Table 1. Participating labs that submitted results in the rapid starch test.

American Sugar Refining, Inc., Baltimore Refinery, Baltimore, Maryland
American Sugar Refining, Inc., Chalmette Refinery, Chalmette, Louisiana
Audubon Sugar Institute, LSU, Baton Rouge, Louisiana
C&H Sugar Refinery, Crockett, California
Colonial Sugars, Gramercy, Louisiana
Cora Texas Manufacturing Company, White Castle, Louisiana
Iberia Sugar Coop, New Iberia, Louisiana
Okeelanta Corporation, South Bay, Florida
Raceland Sugar Company, Raceland, Louisiana
Rio Grande Valley Sugar Growers, Inc., Santa Rosa, Texas
Savannah Sugar Refining Company, Savannah, Georgia
Sugar Cane Growers Cooperative of Florida, Belle Glade, Florida
Sugar Processing Research Institute, Inc., New Orleans, Louisiana
United States Sugar Corp., Clewiston, Florida

Raw sugars for the test were obtained from various participating companies and from SPRI stocks. Samples used in the test consisted of six raw sugars having a wide range of starch concentrations, yet with similar colors, so that the blind duplicates could not be readily discerned. Each participant received 12 packets of sugar, 50 g per packet, each with a 3-digit identification code. The samples were distributed as blind duplicates.

Along with the 12 samples, participants received the instructions for the method to determine starch in raw sugar, the method for developing the standard starch calibration curve, a reporting form, a small amount of standard starch and two raw sugar practice samples with the known starch values provided. Participants were given the option to use the standard starch provided (for convenience purposes only) or to use their own starch. One of the laboratories (Lab 6) used their own starch. Only one analysis per sample was requested.

The results were statistically analyzed using the AOAC Blind Duplicates V1_5 program for determining the statistics of collaborative tests. This package uses the IUPAC protocol for analyzing the results of collaborative studies.

RESULTS

Practice Samples

Table 2 shows the results of the practice samples and the moisture in starch obtained by each lab. Sample A was provided with a given value of 187 ± 5 ppm starch based on replicated results by two analysts at SPRI. When all the lab results are included, Sample A averaged 190 ± 20 ppm starch. If the high result of Lab 5 is eliminated, Sample A averaged 186 ± 14 . When all lab results are included for Sample B, it averaged 372 ± 42 ppm, compared to the 372 ± 18 provided by SPRI. When the high result of Lab 5 and the low result of Lab 9 were eliminated, Sample B averaged 372 ± 24 . These results show good agreement among the participating laboratories, and are in agreement with the reproducibility results obtained in this test.

Table 2. Moisture in starch and results of practice samples

Lab No.	% Moisture	Used Starch Provided by SPRI	Sample A (187 ± 5)	Sample B (372 ± 18)
1	5.16	Yes	174	357
2	5.17	Yes	198	379
3	3.6	Yes	203	415
4	5.3	Yes	181	354
5	5.4	Yes	241	465
6	9.43	No	176	338
7	5.48	Yes	183	359
8	5.18	Yes	181	370
9	4.05	Yes	169	281
10	3.95	Yes	186	376
11	4.11	Yes	184	393
12	4.06	Yes	195	395
13	4.038	Yes	219	388
14	4.30	Yes	175	339
Mean	4.60 ± 0.68*	--	186 ± 14.**	372 ± 24.**

*Does not include the moisture determined by Lab 6, which provided its own starch for calibration. Shaded cells indicate starch values that are significantly higher or lower than the value provided by SPRI.

** Mean does not include values in shaded cells.

Summary of Practice Sample Results:

Sample A

Mean A(all) = 190 \pm 20 (C.V. = 10.5 %)

Mean A (-lab 5) = 186 ± 14 (C.V. = 7.52 %)

Removed highest lab (#5)

Sample B

Mean B (all) = 372 ± 42 (C.V. = 11.3 %)

Mean B (-labs 5, 9) = 372 ± 24 (C.V. = 6.45 %)

Removed highest & lowest labs (#5,9)

Collaborative Test Results

Table 3 shows the raw data received from the participants. Cochran outliers are highlighted in grey cells and Grubbs outliers are shown as bold and underlined.

Outliers. Grey cells (in Table 3) show Cochran outliers. Cochran outliers are results for which the repeatability (results from two identical samples in a laboratory) is poor compared to the rest of the results. We were not able to ascertain the reason for the wide variability in the Lab 7 Cochran outliers, as in each case, one of the results was in agreement with the accepted results. Because Lab 7 results were good on the practice samples, and 6 out of the 12 results were in agreement with the other results, the laboratory was retained for determining the statistics. (It was subsequently determined that Lab 7 had used two different analysts to run the samples, which probably explains the poor repeatability within the blind sample duplicates.)

Bold and underlined cells in Table 3 show the Grubbs outliers. Grubbs outliers are those labs whose results contribute the most to a high standard deviation because of having the highest or lowest average results, based on critical values. The Grubbs outliers for Lab 7 for the Florida-1 and Louisiana sugar are obvious even without statistical analysis. However, the repeatability of Lab 7 for both of these samples was good, which explains why they were not tagged as Cochran outliers.

Precision. In this study, the number of labs eliminated from the statistics due to outliers did not exceed the IUPAC guidelines (labs removed should not exceed 2 labs out of 9, or 22.2% of the labs). For a study including 14 laboratories, not more than 3 labs should be removed (21.4%).

Summary Statistics. Table 4 shows the results of the statistical analysis. HORRAT is the Horwitz Ratio, a value which helps to evaluate the RSD(R)% (reproducibility relative standard deviation %). Since the acceptable variability of a test varies with the concentration (higher variability is expected with lower concentrations), the HORRAT is the ratio of the RSD(R)% of the test and of the expected RSD(R)% for that concentration. An ideal outcome is HORRAT ≤ 1.0 . For evaluation purposes, the HORRAT should not exceed 2.00. The Paraguay sugar fell slightly outside of this range. Both AOAC and ICUMSA have accepted studies in which the HORRAT is slightly above 2, as long as the over-all test statistics are acceptable.

DISCUSSION

Based on the data in Table 4, this test met the criteria for a validated method.

The standard IUPAC/AOAC protocol does not provide for a way to determine one over-all repeatability and reproducibility value for a collaborative test, such as by taking the means of the results. An additional test to compare variances, Bartlett's test, must be done. If the variances are homogenous across the samples, then they can be averaged. Bartlett's test on this data set showed that the variances were not homogenous, so averaging could not be done.

Therefore, since the data in Table 4 show that the RSD(r) ranged from 2.18 to 6.07%, it is possible to say that a single analyst would expect to obtain a starch value in raw sugar that would not exceed this percentage by more than 2.8 times (the repeatability value, r). In reality, the analyst should expect to repeat himself at a value much closer to the RSD(r) range -- that is, somewhere between 2 - 6%.

In a similar manner, the RSD(R) ranged from 5.60% to 18%, with higher variations for lower concentrations of starch. Based on the results for the practice samples, the variation for all labs was on the order of 10.5% to 11.3%, without removing any labs, values that would correspond to the RSD(R) of a collaborative test, indicating that the reproducibility value (as a percent) would be in the range of 10 - 11%.

Using a percentage to evaluate results, as is done in proficiency testing, which uses Robust Statistics, provides a more intuitively satisfactory evaluation of a result. In this type of testing, an acceptable degree of variation, for example, 5% within a lab and 10% between labs may be chosen to evaluate the performance of analysts and laboratories.

All of the labs, except for SPRI, were inexperienced with the method, could have caused an increase in the variability and could have led to a higher relative standard deviation of the reproducibility. Nevertheless, the reproducibility fell within the guidelines for five out of the six sugars, and in the sixth sugar was only slightly outside the guidelines.

Because Lab 7 results were good on the practice samples, and 6 out of the 12 results were in agreement with the other results, the laboratory was retained for determining the statistics. The Grubbs outliers for Lab 7 for the Florida-1 and Louisiana sugar are obvious even without statistical analysis. However, the repeatability of Lab 7 for both of these samples was good, which explains why they were not tagged as Cochran outliers. The high results from this laboratory were consistently higher by a factor of about 2.7 from the average results of the rest of the test. Their other values were consistently within 90% of the average test results. This indicated to us that the analyses could have been done in two separate groups (different days, different analysts, different equipment?) or that something had changed during testing. As mentioned, there were two analysts.

CONCLUSION

It is concluded that the results of the collaborative test meet the precision requirements for a validated method. The repeatability is in the range of 2.5-6.0% and the reproducibility is in the range of 10-11%. This test could be used in mill and refinery laboratories to determine the content of starch in raw sugar.

It is recommended that ICUMSA make this a Tentative method for the determination of starch in raw sugar. At the April 2004 ICUMSA Interim Meeting, this method was given Tentative standing as an ICUMSA Method.

ACKNOWLEDGMENTS

All of the participants in the test are thanked for their hard work in completing the testing in a timely fashion. Dr. Margaret Nemeth, statistician, is thanked for helpful discussions and for calculating the Bartlett's test statistics.

Table 3. Raw data of the collaborative study for rapid SPR1 method for starch determination in raw sugar, showing outliers.

Lab	Paraguay		Guatemala		Florida-1		Philippines		Louisiana		Florida-2	
	600	421	114	333	632	506	282	702	741	968	320	188
1	101	103	174	176	233	229	262	271	370	370	739	735
2	115	99	199	184	227	215	282	290	378	367	722	700
3	113	114	212	218	268	262	308	315	388	384	794	791
4	94	91	175	168	215	216	233	251	331	327	706	703
5	178	<u>205</u>	239	279	306	315	379	330	428	414	796	771
6	91	89	173	175	210	218	251	261	311	310	678	664
7	283	93	164	170	<u>663</u>	<u>654</u>	254	780	<u>1050</u>	<u>1044</u>	711	683
8	111	109	191	196	232	228	279	289	382	381	736	721
9	<u>52</u>	<u>73</u>	180	178	185	183	236	208	336	275	630	727
10	118	114	180	180	251	252	280	290	392	391	788	783
11	108	115	206	206	233	231	291	273	384	387	739	729
12	161	136	211	199	273	251	245	300	413	375	728	746
13	142	145	218	225	260	257	318	308	399	411	814	754
14	93	92	167	163	213	217	250	251	305	309	710	693

Grey cells = Cochran outlier (maximum within lab variance)
Bold and underlined cells = Grubbs outlier (lab or labs that are)

grosses either (due to rates that contribute most to high standard deviation), because of highest or lowest average)

Table 4. Results of starch collaborative study, with outliers removed, as described in the text.

Sample	Paraguay	Guatemala	Florida-1	Philippines	Louisiana	Florida-2
Labs used	11	13	13	13	12	13
Labs removed	3	1	1	1	2	1
Mean	112	188	238	279	371	736
s(r)	6.7	4.8	5.8	16.9	9.0	16.0
s(R)	19.8	19.1	32.1	36.4	37.1	41.2
RSD(r) %	5.98	2.54	2.44	6.07	2.44	2.18
RSD (R) %	17.79	10.18	13.49	13.06	9.99	5.60
r	19	13	16	47	25	45
R	56	54	90	102	104	115
HORRAT	2.26	1.40	1.92	1.90	1.52	0.94

r = repeatability value, $2.8 \times S(r)$

(The amount by which 2 separate determinations on the same sample by an analyst within a laboratory should agree with each (+/-) other 95% of the time.)

R = reproducibility value, $2.8 \times S(R)$

(The amount by which 2 separate determinations conducted in different laboratories on identical materials should agree (+/-) with each other 95% of the time.)

APPLICATION OF A NEW GC-MS METHOD FOR DETERMINING ESTER CONTENTS FOLLOWING ALKALINE OR ENZYMATIC HYDROLYSIS OF SUGAR BEET PULP AND PECTIN

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ABSTRACT

A new gas chromatography method with selective mass spectrometry detection is reported that applies three innovations for analysis of pectin esters – 1) stable deuterated isotopomers, 2) Carboxen-PDMS solid-phase microextraction fibers, and 3) headspace sampling – and separations based on a PoraPLOT Q capillary column. This method provides for simple, fast, and simultaneous determination of methanol and acetic acid released by pectin hydrolysis, and it can be applied to other classes of similarly esterified polysaccharides. This method has been further demonstrated as capable of detecting and simultaneously measuring enzyme activities of pectin acylesterase and methylesterase. Finally, this method provides an important new analytical technology for use in research directed to developing new bioproducts from sugar beet cell wall polysaccharides and for monitoring enzyme activities in food and beverage processing.

INTRODUCTION

The U.S. beet sugar industry generates about 2 million tons dry beet pulp annually (Grohmann and Bothast, 1994). It is sold as a nutritious animal feed (ca. \$85/ton), providing a means for its disposal and recovery of energy cost inputs required to dry the material for storage and shipping. Beet pulp represents an enormous and largely untapped source of valuable structural polysaccharides that could be recovered and used in food, pharmaceutical, and industrial applications. The value of such bioproducts can exceed \$10,000/ton. The development of higher-value co-products from pulp could therefore help improve profitability of the beet sugar industry by adding new value to the crop. This has been addressed previously at the SPRI Conference (Thibault and Bonnin, 2000).

Pectin is a complex cell wall polysaccharide, representing a major fraction of sugar beet pulp (>20% dry weight) (McCready, 1966; Marry et al., 2000). Unique structural features of beet pectin include high content of associated arabinans and acetylestes and the presence of ferulic acid esters, which may form dimer cross-linking structures in the cell wall. These structural features can be exploited for developing new functional uses as binders and stabilizers, chelators and emulsifiers, films and specialty fibers, and viscosity modifiers. Many of sugar beet pectin's functional properties are influenced by chemical decorations along the polysaccharide backbone such as methyl- and acetylestes. Enzymes can be used as an efficient means to remove these esters or to modify their distribution patterns, improving pectin's functionality for a target application (Williamson et al., 1998; Savary et al., 2003).

Improved analytic methods are needed for the specific and sensitive determination of the ester decorations in sugar beet pulp and pectin. Current standard methodologies for methanol and acetic acid are labor-intensive, time-consuming, and require large sample sizes. We have recently reported a simple, fast, and direct gas chromatography procedure for the determination of methanol and acetic acid contents in pectin and other cell wall polysaccharides (Savary and Nuñez, 2003). Coupled with a mass spectrometer, this method takes advantage of commercially available deuterated isotopomers for use as internal standards and a suitable solid-phase microextraction (SPME) fiber for headspace sampling. The simplicity and sensitivity of the method is such that it can also be applied for detecting and quantifying corresponding esterase activities upon treatment of polysaccharide substrates. This method and its application is presented here. Further details and figures were provided in the poster presented at this conference, and they can be obtained in the original report (Savary and Nuñez, 2003).

METHOD DETAILS

The analysis is calibrated for 1 mg of pectin, pulp/cell-wall, or other ester-modified polysaccharide delivered to a sample vial. Residual solvents in polysaccharide samples are removed prior to analysis by dissolution in water then lyophilizing. Ester linkages are hydrolyzed by 0.200 M NaOH in a septum-sealed vial containing internal standards (2.00 μ mol d_3 -MeOH and 0.500 μ mol or 0.050 μ mol d_3 -HOAc) for 1 hr at 40°C. Samples (0.500 mL) are then acidified to ca. pH 2.0 with an equal volume of 0.40 M H₂SO₄. Enzyme-treated materials are similarly prepared but sampled at various time intervals, with the reaction stopped by acid addition. Headspace volatiles in vials are sampled with a Carboxen-PDMS SPME fiber (Supelco) by piercing the vial septum with the fiber assembly, exposing the fiber for 15 min at 40°C., and transfer of the fiber assembly to the GC (Hewlett-Packard 5890 Series II Plus) injector for desorption (splitless, 300°C). Separations are subsequently performed with a Chrompak PoraPLOT Q capillary column (40°C to 250°C at 50°C/min). The Mass Selective Detector is run in electron impact ionization mode (70eV) with data collected at selected ion signals at 1.2 scans/s. Base ion pairs used are *m/z* 29 and 30d for methanol and d_3 -methanol, respectively, and *m/z* 43 and 46d for acetic acid and d_3 -acetic acid, respectively.

Method Evaluation

Based on the manufacturer's recommendation, Carbowax-DVB and Carboxen-PDMS SPME fibers were compared for adsorption of methanol and acetic acid in the headspace of vials. The Carboxen-PDMS provided a better than 10-fold greater recovery for both analytes, and this was significantly improved for acetic acid by further heating the vials to 40°C. The PoraPLOT Q column provided good resolution and peak symmetry for both analytes (see Figure 1). Other columns, including PoraBOND Q, resulted in extensive tailing of acetic acid or insufficient retention of methanol. Calibration curves were constructed by determining the peak area ratios of the base ions and plotting the ratios (unlabelled/isotopomer) of areas vs. the concentration ratios (data fitted by second order polynomial gave r^2 of 0.99 for both analytes).

Methanol and acetic acid contents were determined for three representative samples each from fruit and vegetable pectins. Values determined ranged from a high of 3.70 μmol methanol/mg methylated lime pectin and 0.440 μmol acetic acid/mg sugar beet pectin to lows of 0.427 μmol methanol/mg *Aloe* pectin and 0.018 μmol acetic acid/mg methylated lime pectin. Comparison of contents for a commercial sugar beet and citrus pectin are provided in Table 1. The methanol contents determined by this GC-MS method match those determinations provided by the manufacturers using the Food Codex method. The acetic acid contents determined for the sugar beet pectin appear to be significantly lower than that determined using the coupled enzyme assay. The difference may be due to lower stability of acetyl esters during storage or to much lower precision of the enzyme assay. Since this GC-MS method is based on a direct physical determination, it provides a more reliable and accurate determination, and thus provides a new "gold standard" for measuring methanol and acetic acid.

Table 1. Methanol and acetic acid contents in commercial pectins, comparing GC-MS method with manufacturers' determinations.

Composition	Content by GC-MS ($\mu\text{mol}/\text{mg}$ pectin \pm S.D.)	GC-MS (% \pm S.D.) ¹	Manuf. (%)
SUGAR BEET			
Methoxyl content	1.87 \pm 0.08	6.0 \pm 0.4	6.2
Acetyl content	0.807 \pm 0.046	2.5 \pm 0.1	3.5 ²
Degree Methylation ³		53.1 \pm 3.6	55.4
Degree Acetylation		12.6 \pm 0.7	16.6
CITRUS			
Methoxyl content	2.59 \pm 0.10	8.3 \pm 0.3	8.1
Acetyl content	0.036 \pm 0.005	0.2 \pm 0.03	N.D. ⁴
Degree Methylation		63.0 \pm 0.8	61.8
Degree Acetylation		1.2	N.D.

¹Percent content as mg per mg pectin.

²Determined by coupled enzyme assay kit. Precision or reproducibility was not provided.

³Percent mole ratio to anhydrogalacturonic acid.

⁴N.D., not determined.

We have determined this method can be extended for use in detecting and measuring both methyl- and acetylesterase activities. The chromatogram in Figure 1 represents methanol and acetic acid released by enzyme action on sugar beet pectin. Similarly, we screened through a large set of various commercial enzyme preparations using sugar beet pulp and pectin, and we determined both pectin methylesterase and acetylesterase activities are common. We are now seeking to transfer this analytical technology to industry, where we believe it will be useful in quality control in food and beverage processing where residual esterase activities may degrade pectin functionality in products.

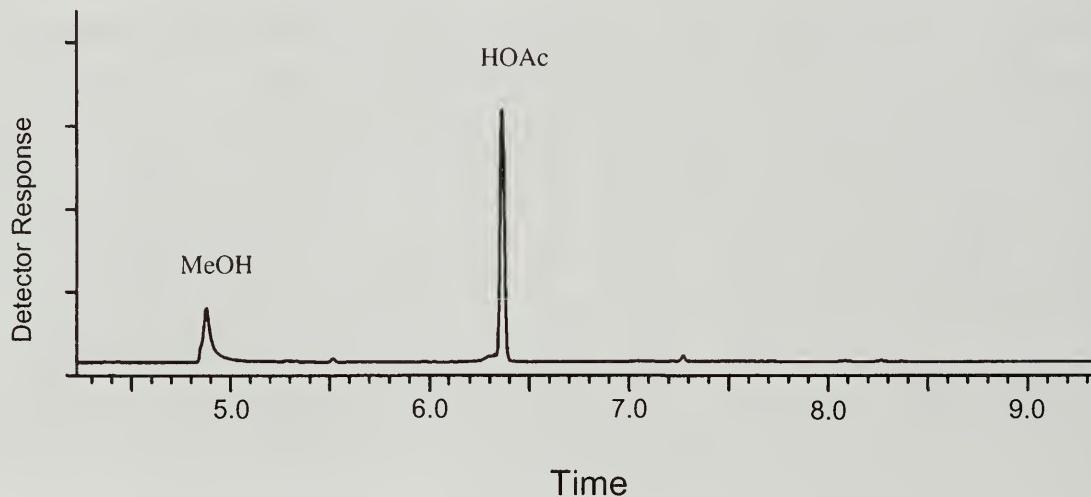


Figure 1. Chromatogram of methanol and acetic acid released from sugar beet pectin esters by action of corresponding enzyme activities and separated on PoraPLOT Q capillary column.

Features of this Method

- Minimal sample consumption (1.00 mg) and simple preparation.
- Effective headspace sampling with the Carboxen-PDMS SPME fiber.
- Suitable retention times, resolution and peak symmetry for methanol and acetic acid using a PoraPLOT Q capillary column.
- Highly accurate quantification by using stable deuterated isotopomers as internal standards.
- Applicable to fruit or vegetable pectins, whole cell wall samples, or other classes of esterified polysaccharides.
- Detection and quantification of corresponding esterase activities.
- Amenable for industrial quality control applications for detecting residual activities of processing enzymes.

ACKNOWLEDGEMENT

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Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

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STUDIES ON BAGASSE FLY ASH AS AN ADSORBENT FOR WASTE MATERIALS

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ABSTRACT

Bagasse fly ash, the material that is carried up and through the generating bank and into the scrubbers after bagasse is burned in the boilers during cane sugar processing, is considered a waste product, with few commercial uses. Recent literature has shown that fly ash may have the potential for use as an adsorbent trap for a variety of organic and inorganic materials, such as pesticides, dyes and heavy metals, making way for a new value added product for the sugarcane industry. In this study, the composition of fly ash from two harvest seasons in Louisiana was determined and its structure examined by microscopy. We measured its ability to absorb textile dye waste, heavy metals and quaternary amines. The fly ash removed 99.8% of textile dyes and removed from 7.5% arsenic to 99.9% chromium, mercury and lead. The composition and microscopic structure of fly ash indicates that removal of compounds is based on the process of chemisorption.

INTRODUCTION

Bagasse fly ash, a portion of the residue remaining after bagasse has been burned in the boilers, is considered a waste product with only a few uses: It has been used as a replacement for bagacillo to improve mud filtration, as an amendment for potting soils and as a housing insulation material in India. However, some recent literature has shown that it may have potential as an adsorbent to trap different organic and inorganic materials, such as pesticides, dyes, and metals, thus providing the opportunity for a new value-added byproduct of the sugar industry.⁽¹⁻⁶⁾ Gupta and co-workers have evaluated the removal of lindane, malathion, DDD, DDE, basic dyes, chromium, cadmium and nickel by bagasse fly ash, with good results.⁽¹⁻⁶⁾

Bagasse fly ash, along with ash from furnace grates and sand, was used to create an artificial wetland in South Africa for effluent treatment. The system was referred to as an ash disposal dam, and was found to remove up to 95% COD.⁽⁷⁾

There is a need for low cost treatment of many waste streams, including food processing streams, feedlot runoff, sewage, textile and distillery waste. A waste product such as bagasse fly ash may have some potential. Treatment of waste streams by bagasse fly ash could be considered green chemistry, since the material does not require extra energy to produce, and since air drying appears to be practical, its final preparation would not require a lot of energy. When exhausted, it could be disposed in a landfill.

Definitions. The residue remaining after bagasse is burned for fuel in the mill consists of furnace ash (also called grate ash, boiler ash, or bottom ash) and fly ash. Fly ash is burnt bagasse that is carried up and through the generating bank and into the scrubbers and thus has much smaller particles than grate ash. Depending on the design and efficiency of the furnaces, more or less fly ash will be produced. In Louisiana, some mills are said to produce more fly ash than grate ash.

Estimated amount of available fly ash. According to Paturau, about 0.3 T of bagasse furnace ash is produced per 100 ton of cane ground.⁽⁸⁾ Today, with the emergence of green cane harvesting, that figure is probably higher. Because at least 2-3% mud and soil comes in with harvested cane, and not all is washed off, the amount of fly ash per season could be 20-30% higher from one year to another. It is estimated that the production of this material can range from 3,000 tons/year for a small factory up to 30,000 tons/year for the largest factories. The presence of field soil (clay), with its ion exchange properties, may enhance adsorbent properties. Figure 1 shows piles of fly ash at a Louisiana mill.



Figure 1. Fly ash piles at a Louisiana factory.

MATERIALS AND METHODS

Samples. Samples of fly ash and boiler ash were obtained from a Louisiana mill in 2002 and 2003. Textile dye waste solution was obtained from Dr. V. Yachmenev, Cotton Chemistry Unit, Southern Regional Research Center. Heavy metal solutions were obtained from Ultra Scientific as 1000 ppm standards in dilute nitric acid, and were diluted to 100 ppm for the experiments.

Sample preparation. At first, based on the literature, the fly ash was treated with hydrogen peroxide to remove organic material,⁽²⁾ but this seemed to degrade performance. It was preferable to have no complicated or expensive treatment of the material. Thus, for subsequent experiments, the only sample preparation consisted of rinsing the fly ash in 3-4 changes of deionized water and oven drying at 80°C. After this treatment, the fly ash consistently produced a pH 8-9 slurry in water. Boiler ash was sieved to obtain particles less than 1 mm in diameter. Fly ash was not sieved.

Treatments. Various types of treatment were tried. Fly ash was used either in small columns (2.5 g or 5 g) or in batch treatment in beakers, with or without heating, and at pH 3, 7 or 9.

Analyses. Removal of dyes was monitored by spectrophotometer scan over the range 400-1000 nm and quantitatively determined at 550 nm. Removal of heavy metals was determined using a Leeman Labs Profile Dual View Inductively Coupled Plasma (ICP) spectrometer, with specific wavelengths for each metal. Benzalkonium chloride was colorimetrically determined by a method used by beverage manufacturers.

RESULTS AND DISCUSSION

Preliminary experiments on textile dyes (see below) showed the boiler ash to have very low activity, so all further experiments were conducted on fly ash only.

Microscopy showed that the particles making up the fly ash were extremely heterogeneous, both in size and appearance, ranging from a few micrometers to about 1 mm. Some particles were agglomerated, creating a porous material, as can be seen in Figures 2 and 3. Incompletely burned plant material, retaining some cellular structure, was also present. The presence of pores and a surface layer of carbon may indicate that the activity of the fly ash in removing various compounds is a chemisorptive process.



Figure 2. Micrograph of fly ash, 150 x.

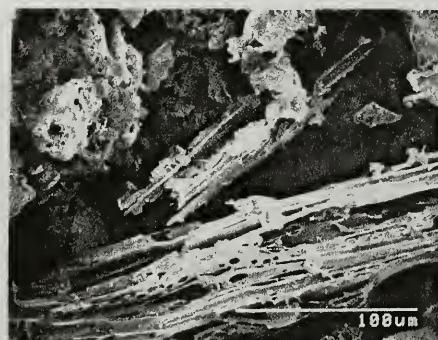


Figure 3. Micrograph of fly ash, 500 x.

Table 1 shows the composition of fly ash from 2002 and 2003, and also compares it to literature values of fly ash from India. Compared to the Indian fly ash, Louisiana fly ash is higher in silicon and has less loss on ignition.

Table 1. Composition of bagasse fly ash

Component, %	2002 sample	2003 sample	Literature ⁽¹⁾
Silicon as SiO ₂	75.15	74.19	61.44
Aluminum as Al ₂ O ₃	9.38	10.08	14.5
Calcium as CaO	1.15	1.09	2.82
Iron as Fe ₂ O ₃	2.65	3.23	4.86
Magnesium as MgO	0.95	1.20	0.71
Loss on Ignition	4.05	8.97	17.12
Surface Area	3.2 m ² /g	28.9 m ² /g	Not reported
Total Carbon	1.63	4.16	Not reported

Several referenced authors treated the fly ash with hydrogen peroxide, to remove traces of organic material, prior to adsorption studies. We compared the action of H₂O₂-treated and not-treated fly ash and found that the action was variable: H₂O₂-treated fly ash removed Coomasie Blue dye while the untreated fly ash had no ability to remove this dye. On the other hand, amido black dye was retained by untreated fly ash but passed through the H₂O₂ unchanged. Untreated fly ash had a capacity of at least 30 mg dye/g fly ash for amido black, an acid dye. All subsequent experiments were done with untreated fly ash.

Textile dye waste. On treating textile waste dye solution (17% Kayacelon reactive blue dye, 0.05% Kayacelon reactive scarlet dye, 1g/l buffer at pH 7.0, 60 g/L sodium sulfite and surfactant), it was noted that at pH 7 and 9, the dyes were not removed by the boiler ash. (These dyes are not pH sensitive.) Figure 4 compares the adsorptive capacity of the boiler ash and the fly ash. As can be seen, the fly ash had a much higher level of performance at all pH levels, with pH 3.0 being the best for all treatments. The best performance was at pH 3.0, with treatment at 55 °C for 1.5 hr, but the performance was almost as good at room temperature for 1.5 hr (96.7% removal compared to 99.8% removal, see Figure 5). When the heat treatment was extended for 3 hr, some of the dye began to desorb, indicating this is an exothermic reaction. Table 2 summarizes the performance of boiler ash and fly ash.

Benzalkonium chloride. Benzalkonium chloride is used as a standard quaternary amine. Quats are often used as biocides in agricultural practice. 20 ml of a 50 ppm solution was treated with 2.5 g and 5.0 g of fly ash at room temperature overnight. The pH was not adjusted, and was 8. The 2.5 g removed 96.1% of the benzalkonium chloride, and the 5 g removed 100%.

Textile Dye Waste Treated with Mill Ash

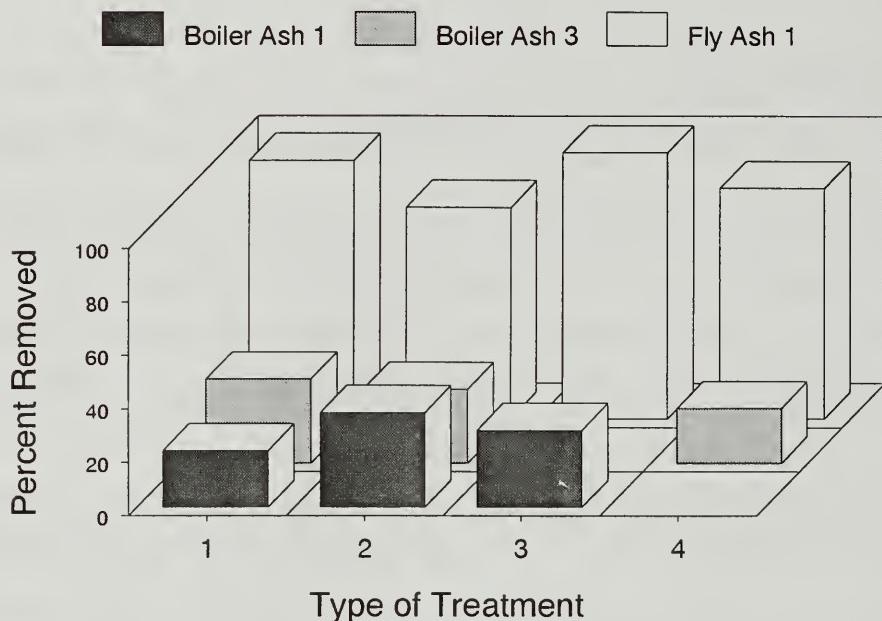


Figure 4. The effect of mill ash on removal of textile waste dye at pH 3.0.

Treatment 1 = Room temperature, 1.5 hours, pH 3

Treatment 2 = Room temperature, 3 hours, pH 3

Treatment 3 = 55 °C, 1.5 hours, pH 3

Treatment 4 = 55 °C, 3 hours, pH 3

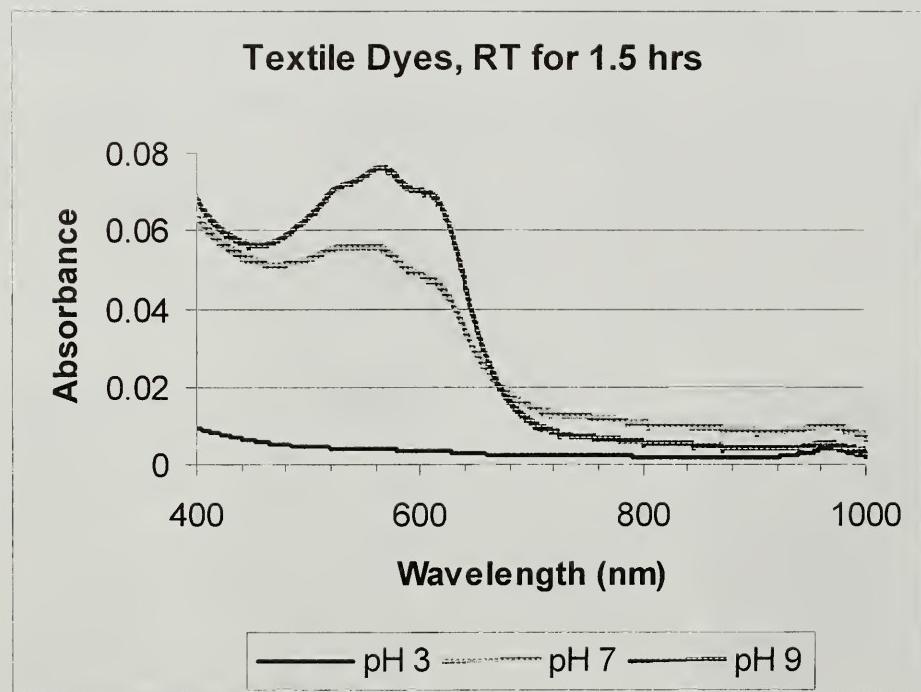


Figure 5. Adsorption profile of textile dyes after treatment with bagasse fly ash at pH 3, 7 and 9.

Table 2. Comparison of boiler ash and fly ash ability to remove textile dye waste.

Reaction Conditions	% Removed								
	Boiler Ash #1			Boiler Ash #2			Fly Ash		
	pH 3	pH 7	pH 9	pH 3	pH 7	pH 9	pH 3	pH 7	pH 9
RT 1.5 hrs	21.3	0	0	31.4	0	0	96.7	55.6	40.1
RT 3 hrs	35.4	0	0	27.6	4.9	3.6	79.3	61.5	35.1
55°C 1.5 hrs	28.9	0	0	0	0	0	99.8	77.0	57.4
55°C 3 hrs	0	0	0	20.5	0.9	0	86.5	57.0	45.7

RT = Room temperature.

Heavy metals. Five common heavy metals of concern were studied, including arsenic (As), cadmium (Cd), chromium (Cr), lead (Pb), and mercury (Hg). 20 ml of 100 ppm metal solutions were treated batchwise with 2.5 g or 5.0 g of fly ash, stirred for a few hours at room temperature and left overnight, after which the samples were analyzed by ICP. Samples from 2002 and 2003 were compared. Figure 6 shows the results for 2.5 g and Figure 7 shows results for 5.0 g. The performance of the fly ash was comparable over the two years. Mercury and lead showed the best sorption efficiency under these conditions, with that of chromium improving from about 50% to almost 100% with a doubling of the quantity of fly ash.

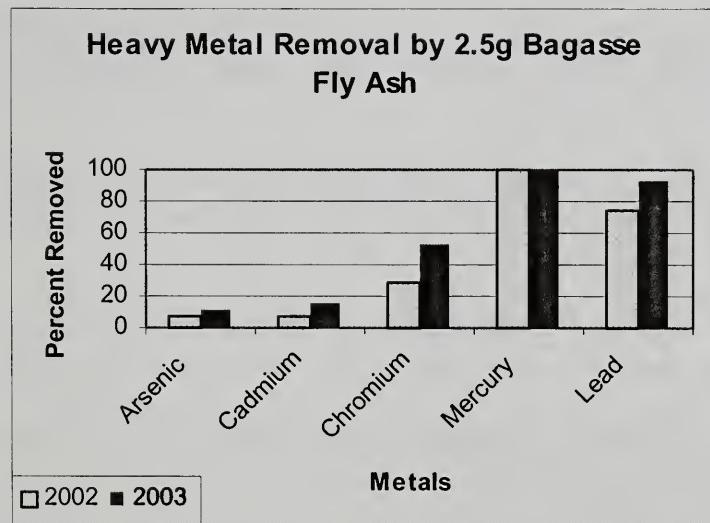


Figure 6. Heavy metal removal by 2.5 g bagasse fly ash.

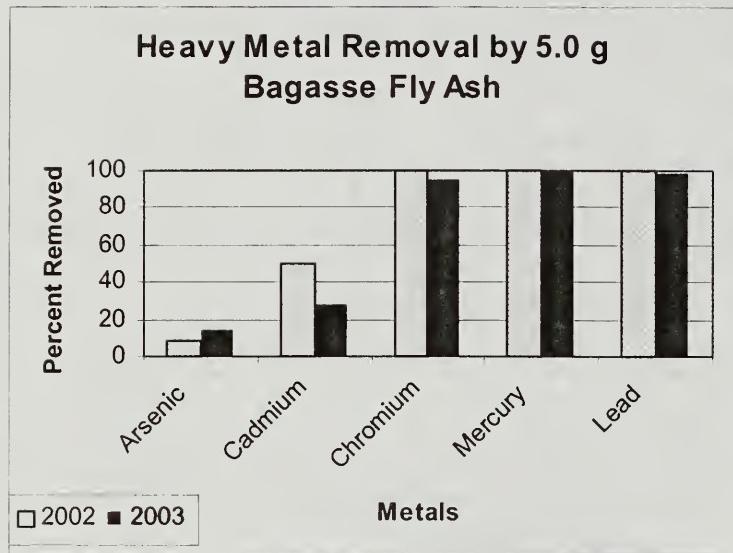


Figure 7. Heavy metal removal by 5.0 g bagasse fly ash.

In conclusion, these results show that bagasse fly ash has the potential to be used as an adsorbent to remove undesirable pollutants. Little pre-treatment is required, although the pH should be optimized for maximum absorbance of target compounds.

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CRITICAL INFLUENCE OF pH ON POLYOL PRODUCTION BY *HANSENULA ANOMALA* IN SUCROSE-BASED MEDIUM

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ABSTRACT

This paper describes utilization of sucrose by the osmophilic yeast *Hansenula anomala* for production of two polyols viz. glycerol and arabitol. The effect of pH and invertase addition on combined polyol yield and productivity by *H. anomala* was investigated. pH 8.0 was found to be optimal, but a substantial amount of sucrose remained unutilized. Though *H. anomala* is known to possess invertase activity, under the conditions of fermentation used, the level of invertase present was not sufficient to invert all sucrose to reducing sugars. Invertase addition resulted in a maximal sugar utilization rate of 2.17 g/l.h at pH 8.0 and a polyol yield of 41.1 % based on total initial sugar.

Key words: Polyols, Arabitol, Glycerol, *H. anomala*, Osmophilic yeast, Sucrose.

INTRODUCTION

Sugar cane and sugar beet processing products viz. molasses, cane/beet juice or crystalline sucrose are renewable raw materials that can be used to develop new fermentation products. Production of glycerol and related polyols viz. arabitol, erythritol, and mannitol by fermentation of renewable sugary raw materials by osmophilic yeast is an interesting research area in yeast biotechnology. Glucose-based fermentation media for polyol production using osmophilic yeast have been reported using *Moniliella tomentosa* var *pollinis* (Burshapers et.

al., 2002a & b), *Candida krusei* (Yongqiang Liu et. al. 2002), *Pichia farinosa* (Vijaikishor and Karanth, 1984 & Bisping et. al., 1990), *Schizosaccharomyces rouxii* (Spencer and Ping, 1957), *Torulopsis magnoliae* (Button et. al., 1966 & Ramchandran and Sulebele, 1979) and *Hansenula anomala* (Spencer and Spencer, 1978). Sucrose being cheaper in developing countries like India, it would be desirable to study the production of polyol from sucrose-based media. Most of the researchers have used glucose-based media. There are very few current reports on use of molasses or juice or even synthetic sucrose media for production of polyol employing osmophilic yeast (Onishi, 1963). Molasses and juice contain many organic and inorganic components and their concentrations vary depending on cane/beet variety, soil characteristics and processing conditions. For the purpose of optimization of fermentation processes it is advisable to start with a synthetic medium with known concentrations of its components. Osmophilic yeast essentially synthesize these polyols under fully aerobic and high osmotic pressure conditions (Yongqiang et. al. 2002, Spencer and Spencer, 1978). Apart from the effect of medium components, process parameters such as pH and dissolved oxygen concentration greatly influence the polyol yield (Vijaikishor and Karanth, 1984, Patil and Sastri, 1994).

In the present investigation, we report on the use of a sucrose-based synthetic medium for production of polyols using *H. anomala* in the pH range 4.0 to 8.5. Externally added invertase was found to facilitate sucrose hydrolysis, resulting in increased polyol yield and productivity.

MATERIALS AND METHODS

H. anomala (NCIM-3341) was maintained on MGYP agar slants and stored at 4°C. The composition of the inoculum development medium used was (g/l): sucrose, 100.0; yeast extract, 5.0; urea, 1.0 and initial pH was adjusted to 6.0 with 0.25 N NaOH. Conical flasks (500 ml) containing 100 ml of this medium were inoculated with a loopful of culture from a fresh slant. After 24 h of growth in an incubator shaker (30°C, 180 rpm), a culture volume corresponding to 10 % (v/v) of main fermenter volume was centrifuged (4000 rpm, 4°C) under aseptic conditions. After discarding the supernatant, the residual cell mass was resuspended in the fermentation medium and used to inoculate the fermenter.

Fermenter-scale experiments: Fermentations were carried out in an automated Chemap laboratory fermenter having a 5 liter capacity, which was equipped with pH and pO₂ electrodes (Ingold). Temperature and agitator speed were maintained at 30°C and 600 rpm, respectively. Sterile airflow was controlled at 0.54 vvm rate to maintain fully aerobic conditions. Foam level was controlled by intermittent addition of silicon (Polymethyl siloxane) based antifoam emulsion (Sigma, A 5758).

Fermentation medium composition was (g/l): sucrose, 300; yeast extract, 2.25; urea, 1.0; casein hydrolysate, 1.0; and MgSO₄.7H₂O, 0.25. The sugar solution was sterilized in situ in the glass fermenter. Other components were sterilized separately and the sterilized nutrients were then pumped aseptically into the fermenter. Before inoculation, pH was adjusted to 6.0 with 0.25 N sterile NaOH and the culture medium was sparged with air to bring the pO₂ value to 100 % saturation. Invertase (β-D-fructofuranosidase, Activity: 30,000 BU/ml) was added at

the time of inoculation and used at a concentration of 0.05 % (w/w of sucrose) whenever required.

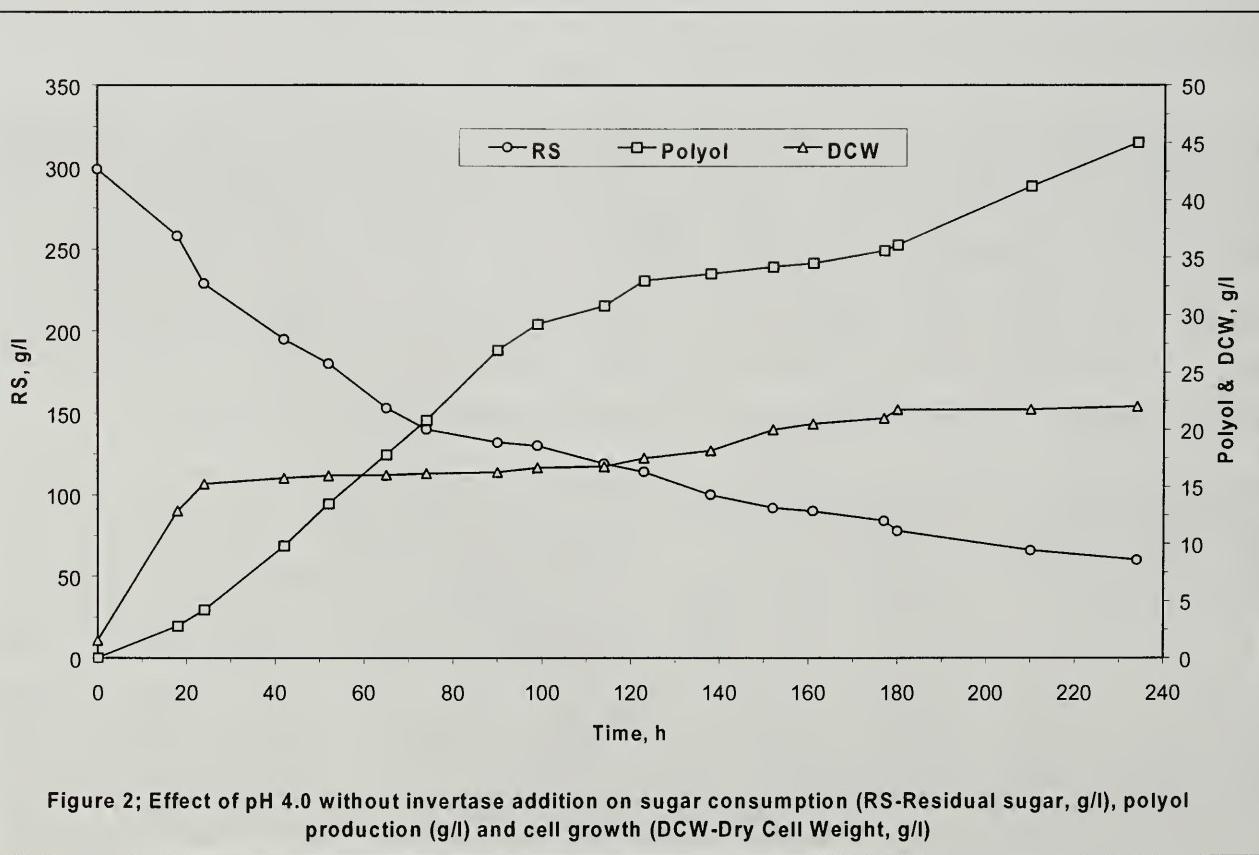
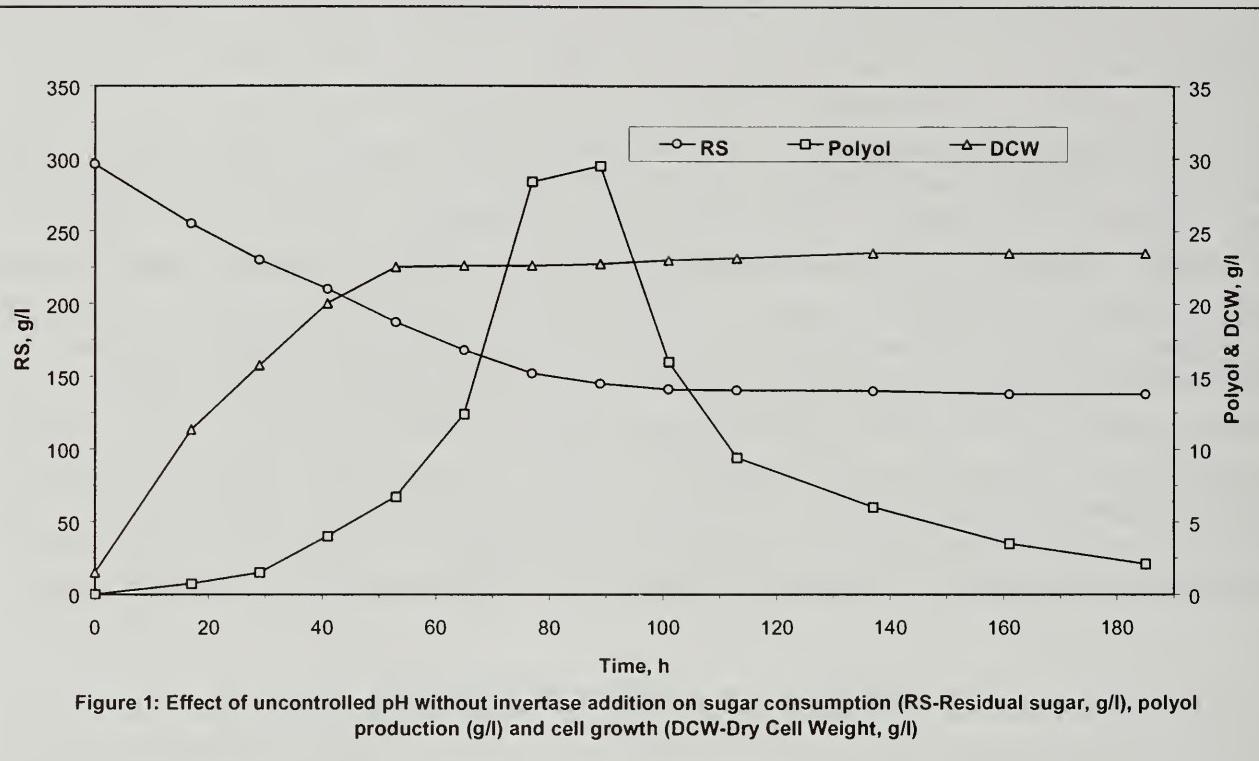
For pH-controlled experiments, pH was adjusted to the required value after 18 h and maintained throughout with automatic addition of sterile 5.0 N Na₂CO₃ solution. Samples were withdrawn aseptically at regular intervals and centrifuged at 4000 rpm to remove cell mass. Supernatant fluids were passed through a 0.22 µm Millipore membrane filter and used for the estimation of total reducing sugar after inversion with HCl and polyol. Total polyol were estimated spectrophotometrically by the chromotropic acid method (Lambert and Neish, 1950) and total reducing sugars by the alkaline copper and arsenomolybdate reagent method (Nelson, 1944). Qualitative TLC of final samples was performed on silica gel plates using an ethanol:acetone:chloroform solvent system (1:1:2). Developed plates were sprayed with Molisch reagent (Furniss, B. S. et. al., 1978) and heated at 150°C for 10 minutes. Distinct coloured spots for polyol appear on a light brown background. Residual wet cell mass after centrifugation was dried in a vacuum oven to constant weight and expressed as DCW, g/l.

RESULTS AND DISCUSSION

The effect of pH on polyols production by *H. anomala* was investigated at uncontrolled pH, pH 4.0, 5.0, 6.0, 7.0, 8.0 and 8.5. Typical time-course profiles for sugar utilization, cell mass generation and total polyol production for uncontrolled pH and pH 4.0 are illustrated in Figures 1 and 2, respectively. Figures 3 and 5 illustrate the effect of invertase addition at pH-8.0 and 8.5 respectively. Figure 4 indicates a profile of variation of pO₂ (% saturation) at pH 8.0 and in the presence of external invertase.

Uncontrolled pH. After rapid growth phase for about 50 h, the cell mass concentration remained almost constant (Figure 1). Sugar utilization was parallel to the growth phase, but continued until about 90 h and was further consumed at a very slow rate. It was observed that when pH was not controlled it dropped to 2.60 in 77 h. Polyol production slowly increased up to about 77 h. A shift in metabolism occurred after 77 h, when the organism stopped utilizing sucrose and started consuming the polyol produced earlier. Maximal polyol and cell mass yield based on the total initial sugars were 10.0 % (at 89 h) and 7.4 % (at 184 h). A substantial amount of sucrose remained unutilized even after 184 h of fermentation.

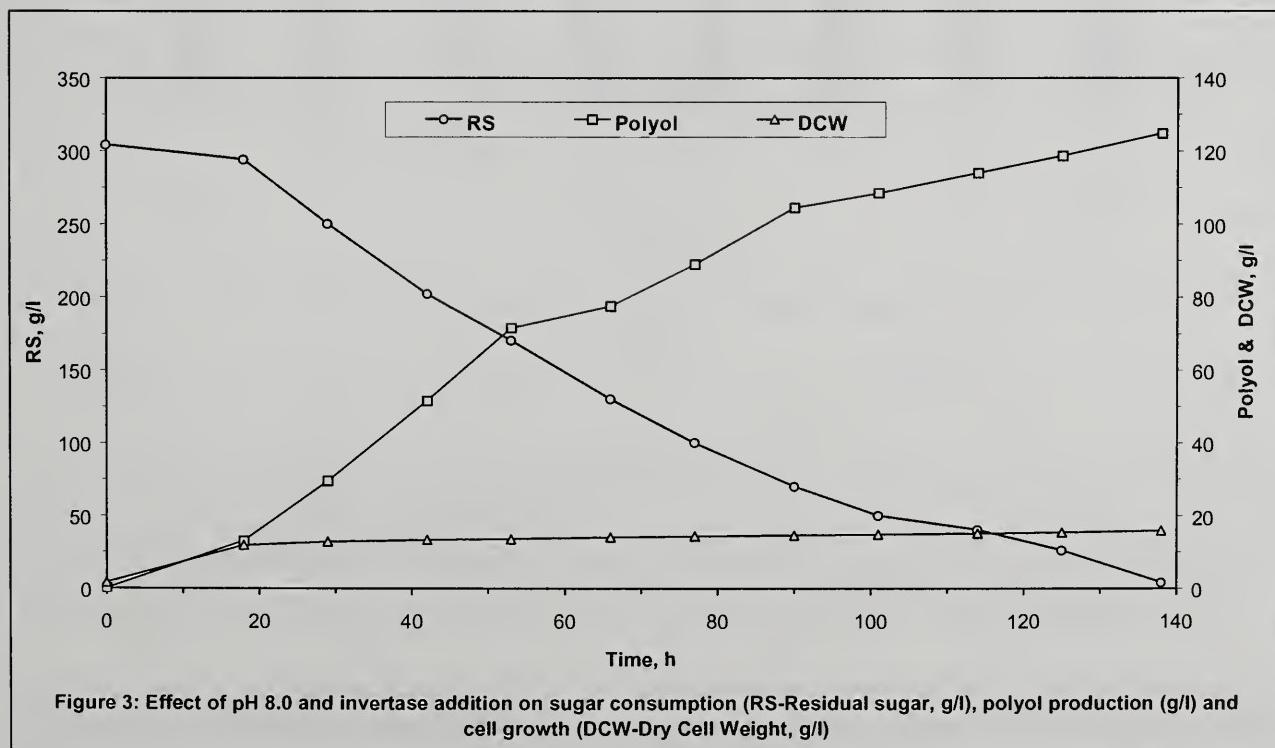
Controlled pH. With all pH controlled experiments, the initial pH before inoculation was adjusted to 6.0 with 0.25 N sterile NaOH. The pH dropped to 2.85 to 2.95 in the first 18 h, which covers the early period of growth. The strategy of adjusting the pH to the required value only after 18 h of growth was adopted. For a controlled pH of 4.0, after the initial phase of 24 h, the increase in cell mass was very slow (Figure 2). Sugar utilization was reasonably fast up to 74 h and slowed down thereafter. Even after 234 h, 6.0 % sugars remained unutilized. Similarly, polyol production increased up to 90-100 h and then declined. Final polyol and cell mass yield reached 15.1 % and 6.8 % at 234 h. Similar trends have been observed with other controlled pH values of 5.0, 6.0, 7.0 and 8.0.



At pH 8.0, the cell mass yield was 6.6 % whereas the polyol yield based on total initial sucrose increased to 21.8 % (Table 1). After 108 h, sucrose utilisation almost stopped, probably because of very weak endogenous invertase activity. Polyol yield based on sugar utilised increased to 41.2 % indicating that a pH of 8.0 favoured polyol production (Table 1).

From a process development point of view, product yield based on total initial sugar is an important criterion. In all the above experiments, sucrose was never completely utilised and, in the later phase of the fermentation, the yeast shifted its metabolism and started consuming polyol. This is contrary to our observations made with glucose (Patil and Sastri, 1996) where the organism began to consume polyol only after all of the sugar had been completely utilised.

We suspected that the endogenous invertase activity of *H. anomala* at pH 8.0 is very weak, which results in a substantial amount of sucrose remaining unutilised. Therefore, invertase was added to the culture to improve sucrose inversion and hexose uptake rate by the yeast. Sugar was almost completely consumed after 138 h (Figure 3). The final polyol yield based on total initial sugar increased to 41.1 % (Table 1). However, cell mass yield decreased to 4.7% when compared to without invertase addition. As described earlier, air flow was maintained constant at 0.54 vvm rate, which resulted in a typical profile of variation of pO₂ (Figure 4). The profile of variation of pO₂ was almost similar to other pH values. Similar trends have also been observed for the case of controlled pH 8.5 with invertase addition (Figure 5). However, complete sucrose utilisation took a longer time (234 h) indicating a slower metabolism at pH 8.5. Though the final polyol yield, based on total initial sucrose was almost the same (41.9 %) as that of pH 8.0 + invertase (41.1 %, Table 1), cell mass productivity (0.054 g/l.h at pH 8.5) was considerably less (0.104 g/l/h at pH 8.0), which indicates the reduced metabolic activity at pH 8.5.



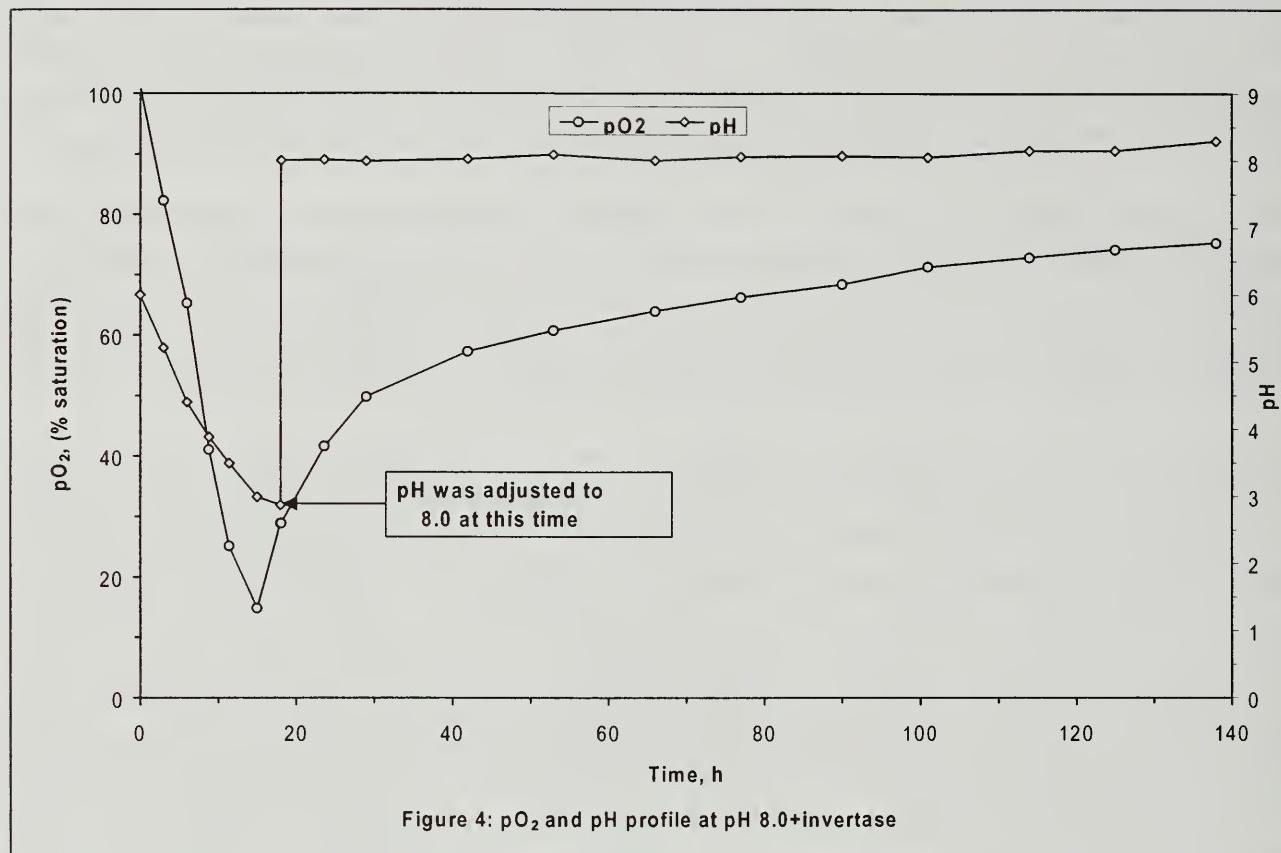


Figure 4: pO_2 and pH profile at pH 8.0+invertase

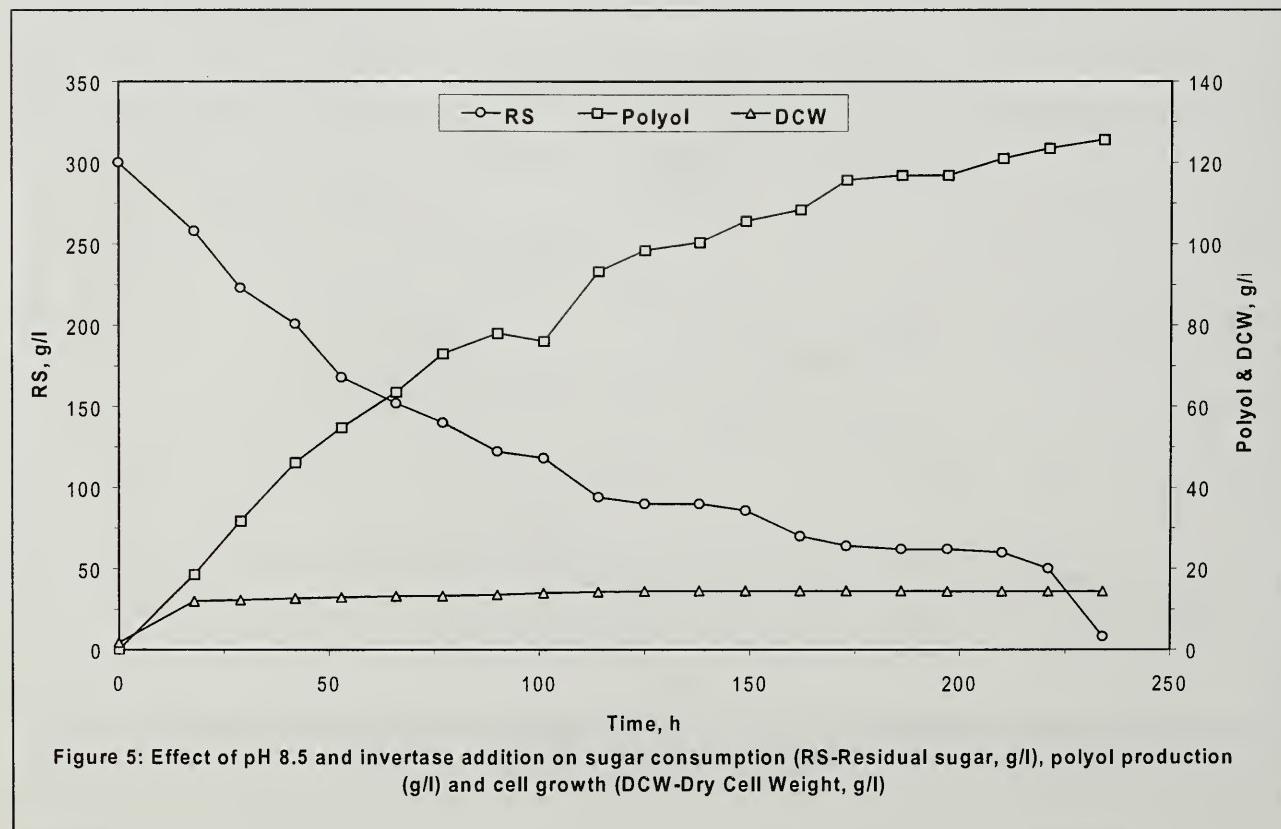
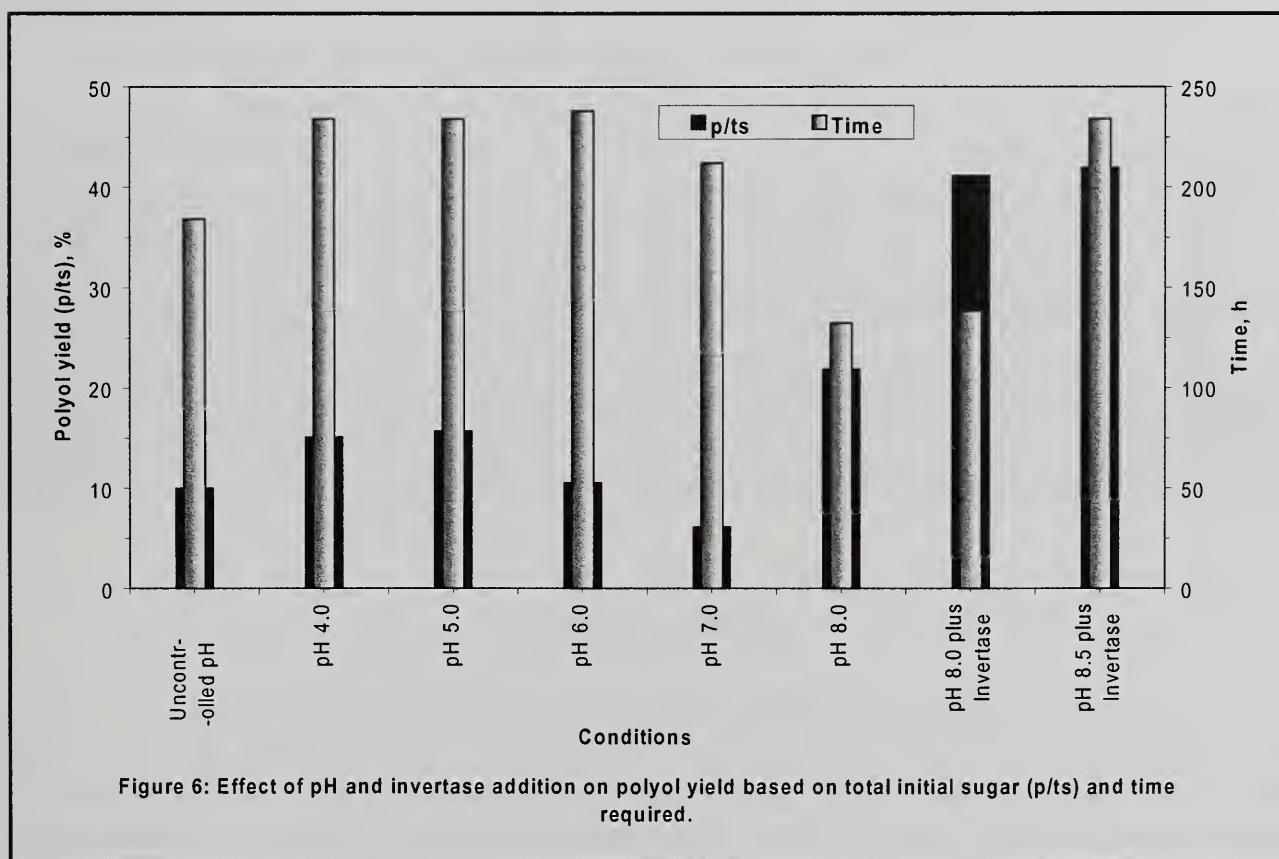


Figure 5: Effect of pH 8.5 and invertase addition on sugar consumption (RS-Residual sugar, g/l), polyol production (g/l) and cell growth (DCW-Dry Cell Weight, g/l)

Final polyol yield based on total initial sugar and the duration of fermentation at different pH values with and without addition of invertase, were compared (Figure 6). Polyol yield is lowest with uncontrolled pH, was maximal at pH 5.0, was significantly reduced at pH 6.0 and pH 7.0 but increased significantly at pH 8.0. One possible explanation for this is fixing of acetaldehyde in the glycolytic pathway under alkaline conditions, thus diverting the sugar and NADH towards glycerol formation (Rehm, 1988; Spencer, 1968). Similar results have been reported with glucose (Vijaikishor and Karanth, 1986; Patil and Sastri, 1996) with complete utilisation of the sugar within about 110 h with a polyol yield of 50 % (based on total initial sugars). With sucrose, *H. anomala* (NCIM-3341) is not able to utilise the sugar completely and substantial amounts of sucrose remain unutilised. At pH 8.0 and without invertase addition, the polyol yield based on total initial sugar and on sucrose consumed was 21.8 % and 41.2%, respectively. This indicated that pH 8.0 is favourable for polyol synthesis but that the endogenous invertase activity is weak, which resulted in incomplete utilisation of sucrose.



With the addition of invertase, sucrose was consumed much more rapidly i.e. within 138 h. At pH 8.5 and in the presence of invertase, the polyol yield based on sucrose utilized was almost the same, but the time required for complete sugar utilization was significantly longer (near 234 h) for complete sugar utilisation. This indicated that pH 8.0 with added invertase is the optimal condition for polyol production by *H. anomala* with sucrose as substrate.

Table 1 shows the comparative final cell mass yields based on total initial sugar and the duration of fermentation at different pH values in presence of invertase. Yeast growth was greater under acidic conditions, but polyol production was reduced. At pH 8.0, the cell mass yield was reduced but polyol production had improved. With addition of invertase, the polyol

yield improved, but the cell mass yield was reduced. At pH 8.5, the cell mass yield was reduced further and the time required to utilise sucrose completely increased. This again indicated that alkaline pH is favourable for polyol production but not for cell growth.

Table 1 also shows the comparative sugar utilisation rates and at different pH values with and without invertase addition. It clearly indicates that pH 8.0 with added invertase provides maximal sugar utilisation followed by pH 8.5 with invertase and then pH 8.0 without invertase. Below pH 7.0, sugar utilisation rate was comparatively longer.

Table 1: Yield and Sucrose utilization

pH	Duration, h	Yield based on sucrose utilized, %		Yield based on total initial sucrose, %		Sucrose utilization rate, g/l.h
		Polyol	Cell mass (DCW)	Polyol	Cell mass (DCW)	
Uncontrolled	184	1.3	13.9	10.0	7.4	0.85
4.0	234	18.8	8.6	15.1	6.8	1.02
5.0	234	17.0	8.4	15.7	7.3	1.11
6.0	238	11.7	7.9	10.5	7.1	1.09
7.0	212	10.0	12.3	6.1	7.7	0.83
8.0	132	41.2	13.2	21.8	6.6	1.21
8.0+Invertase	138	41.6	4.8	41.1	4.7	2.17
8.5+Invertase	234	43.0	4.4	41.9	4.2	1.24

CONCLUSION

This is probably the first report of polyol production with osmophilic yeasts using sucrose-based medium. *H. anomala* is known to have endogenous invertase activity (Kreger-Van Rij, 1984). However, with our strain, and particularly under alkaline conditions, it is not powerful enough to hydrolyse sucrose completely. Addition of invertase has improved sucrose utilization and final polyol yields. A maximal polyol yield of 41.1 % based on total initial sucrose and a sucrose utilisation rate of 2.17 g/l.h has been obtained at pH 8.0 after addition of invertase. However, the final polyol yields reported here are less than those obtained with glucose as substrate (Vijaikishor and Karanth, 1986; Patil and Sastri, 1996). Qualitative thin layer chromatographic analysis of final fermentation broth samples showed that glycerol and arabitol were the only two polyols produced in an approximate ratio of 4:1.

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PROCESS TECHNOLOGICAL AND ECONOMICAL INTEGRATION OF VTIR (TECHNOLOGICAL AND INDUSTRIAL VALUE OF BEET): HARVESTING, WASHING AND PROCESSING

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ABSTRACT

During the 2003/04 campaign (fall and spring sowing) several pilot plant scale experiments that reproduced the factory process from harvested beets to thick juice were conducted with variable beet post-harvest treatment and washing conditions. Data obtained showed an influence of these two factors upon the results of the rest of the process.

On one hand, this study presents and quantifies the effects of some of the parameters of post-harvest treatment and washing on industrial yields for both south and north of Spain beets culture conditions. More precisely, it presents the fluctuations ranges of parameters such as sucrose losses, juice filterability, color and purity of thick juice induced by post harvest factors like storage time, temperature, mechanical damages and calculate the technological and economical repercussions of these factors.

On the other hand, it shows and quantifies the potential increase of beet industrial value (VTIR) that can reasonably be attained through the optimization of both beet post-harvest treatment and beet washing conditions.

INTRODUCTION

Process Technological and Economical Integration. Business opportunities within the well known sugar technology.

Sugar factory: Less than manufacturing. Separating sucrose.

Business opportunities: As near to the beginning of the agro-industrial chain, the higher the benefits and lower the efforts. VTIR equations: Once we established a Spanish way for predicting the process results from the beet composition we identified important opportunities for improving the overall agro-industrial results.

Beet treatment from growing to processing: Harvesting, Cleaning, Transport and logistic, Storage and Washing. This presentation includes the results from 5 different projects: (Analytical scale, Pilot plant scale and Industrial scale) based on 300 trials and 4000 analyzed samples.

Practical philosophy: Profitability of the knowledge (results) will be directly applicable in our Company. We are trying to remain being simple ("sencillos") where we don't need more complex chemical/ biochemical explanations. This presentation therefore should give us one view as clear as possible from the overall beet treatment to the processing results integrating the spring and autumn sown beets. There is a lot of information available published before about these issues to be presented by this paper.

Business opportunity: Competitiveness.

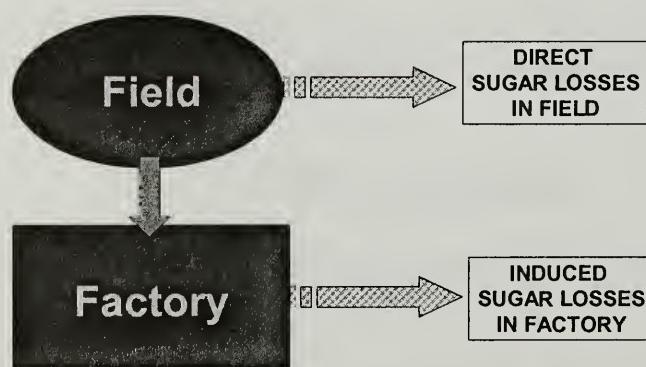
- Campaign duration (80 days/year to 210 days/year)
- Sugar recovery. Industrial; agro-industrial; overall
- Productivity; energy; consumptions (chemicals...).

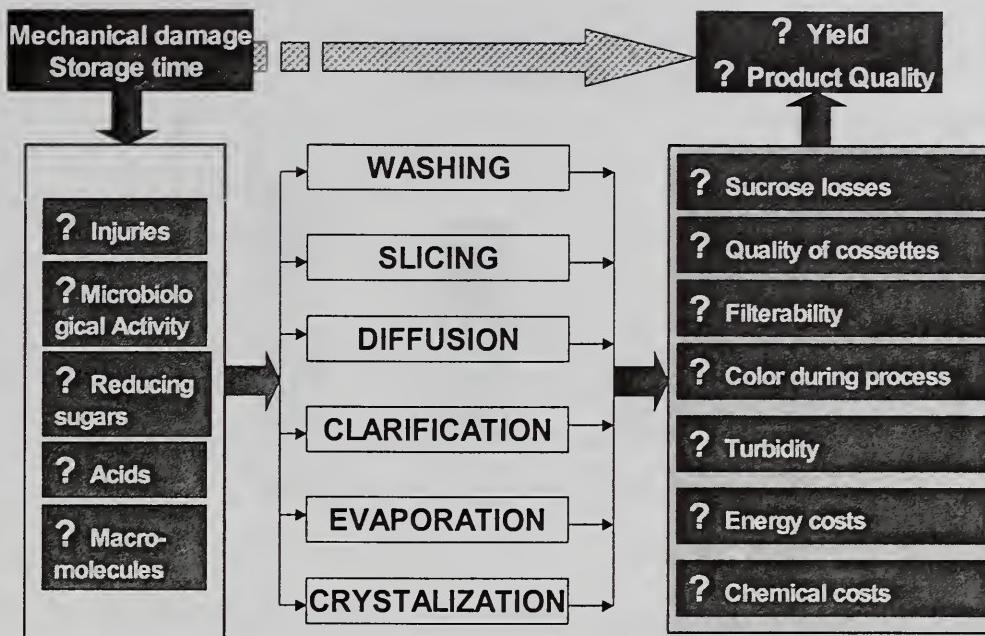
The main industrial indicators:

- Thick juice purity
- Thick juice color
- Effective alkalinity

Beet treatment process results:

- Mechanical damage
- Storage
- Time
- Washing





RESULTS

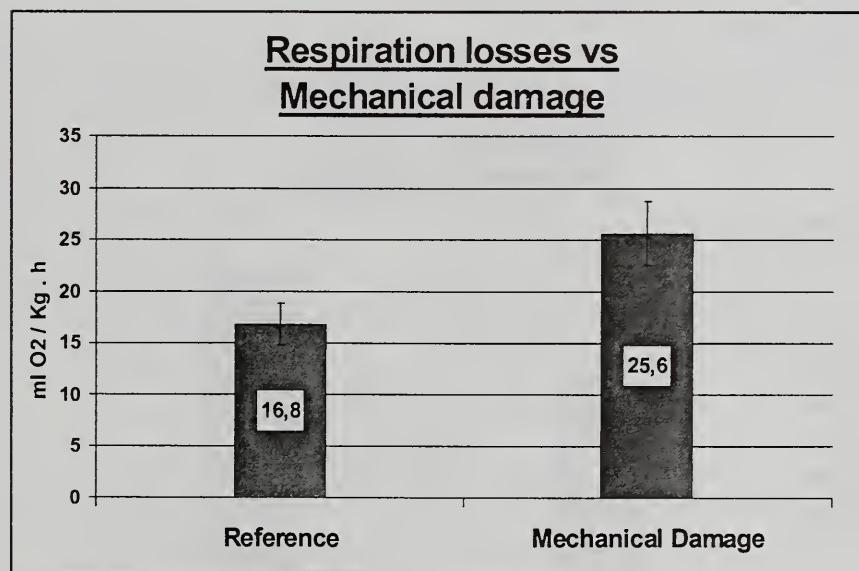


Figure 1. Mechanical damage vs. Respiration (autumn sown beets)

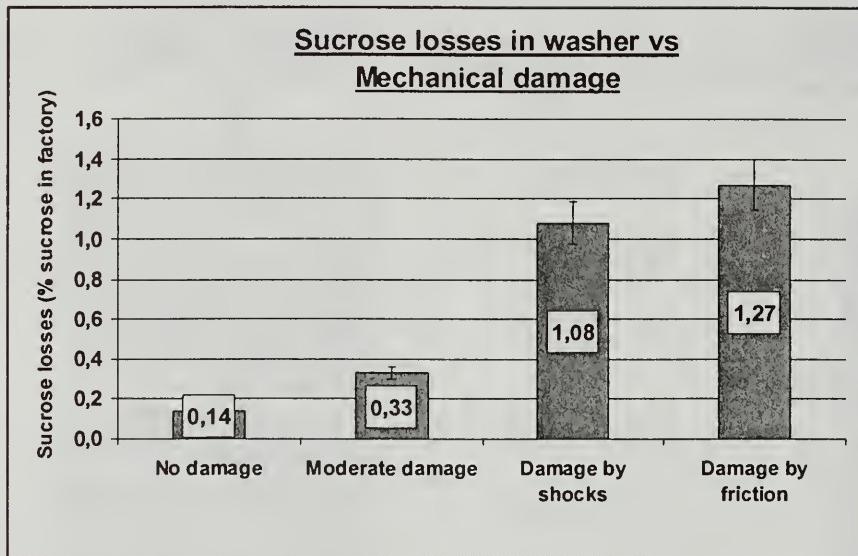


Figure 2. Mechanical damage vs. Washing sugar losses(autumn sown beets)

Sucrose Losses:

- Undamaged beets 0.14% o.S.
- Normal damaged beets 1.4x (0.33% o.S.)
- Highly damaged beets 10x (<1% o.S.)

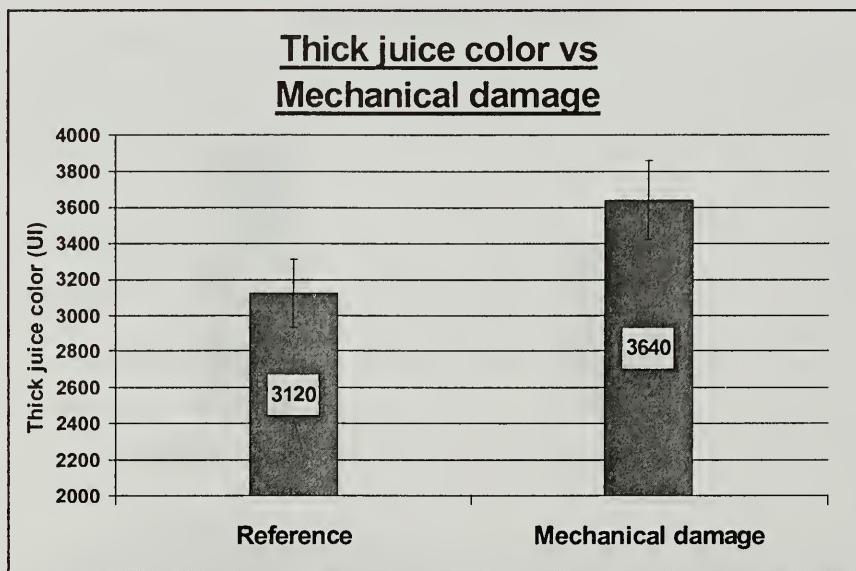


Figure 3. Mechanical damage vs. Thick juice color (autumn sown beets)

Mechanical damage vs. Sugar recovery (autumn sown beets)

- Decrease of the sugar recovery (< 1%)
- Decrease of purity (0,5%)
- Increase of caustic soda consumption (20%)

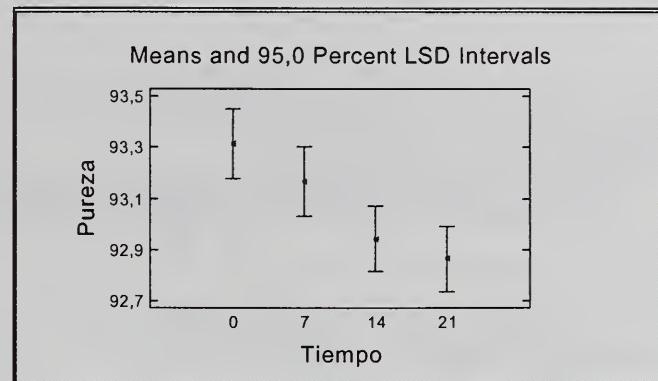


Figure 4. Time (harvesting/slicing) vs. Thick juice purity (autumn sown beets)

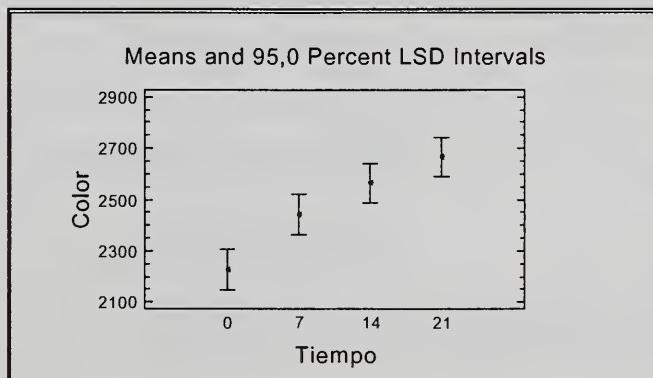


Figure 5. Time (harvesting/slicing) vs. Thick juice color (autumn sown beets)

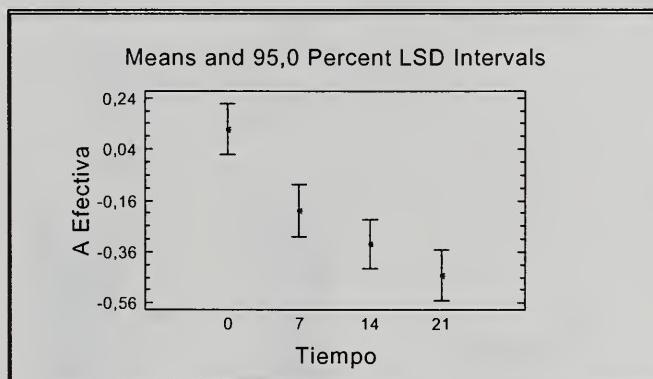


Figure 6. Time (harvesting/slicing) vs. Effective alkalinity (autumn sown beets)

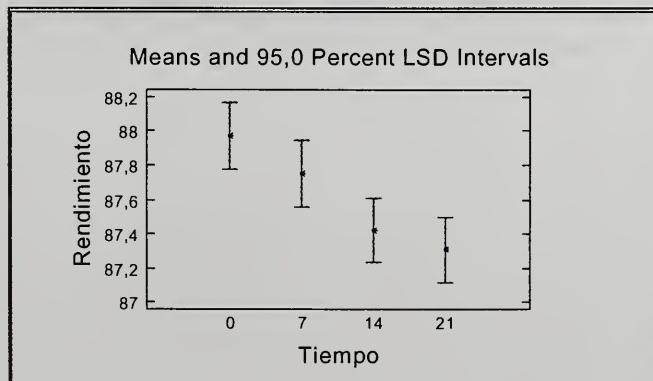


Figure 7. Time (harvesting/slicing) vs. Sugar recovery (autumn sown beets)

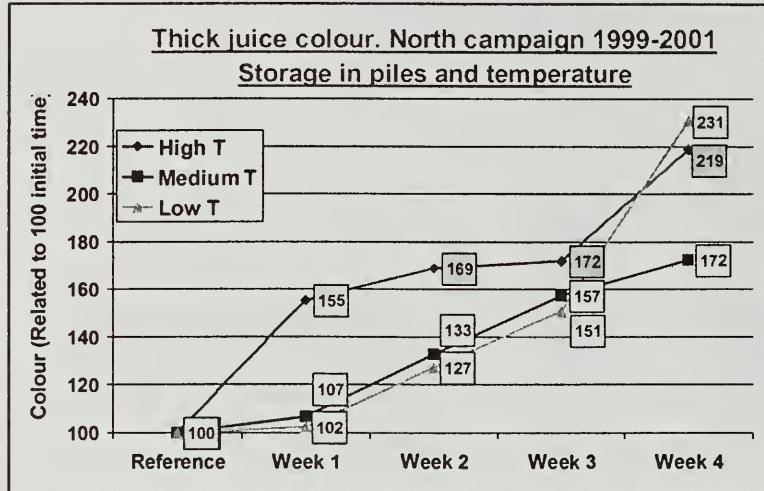


Figure 8. Time (field storage) vs. Thick juice color (spring sown beets)

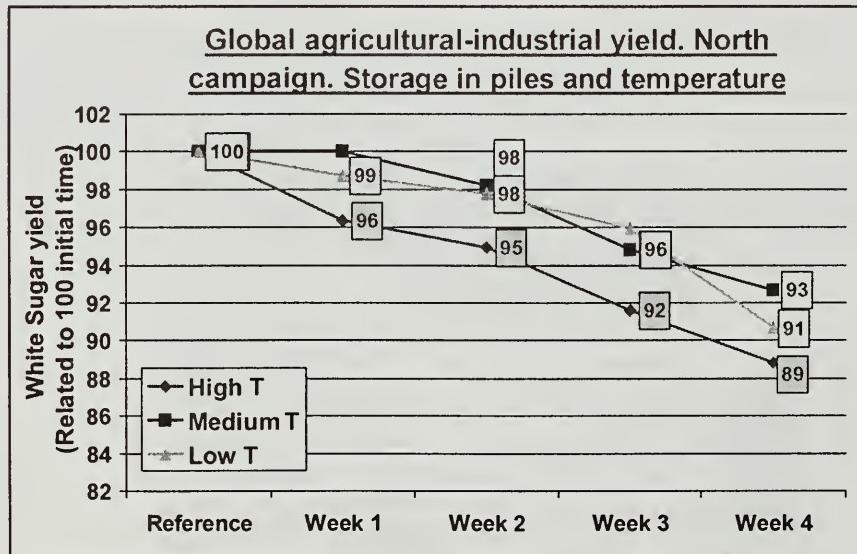
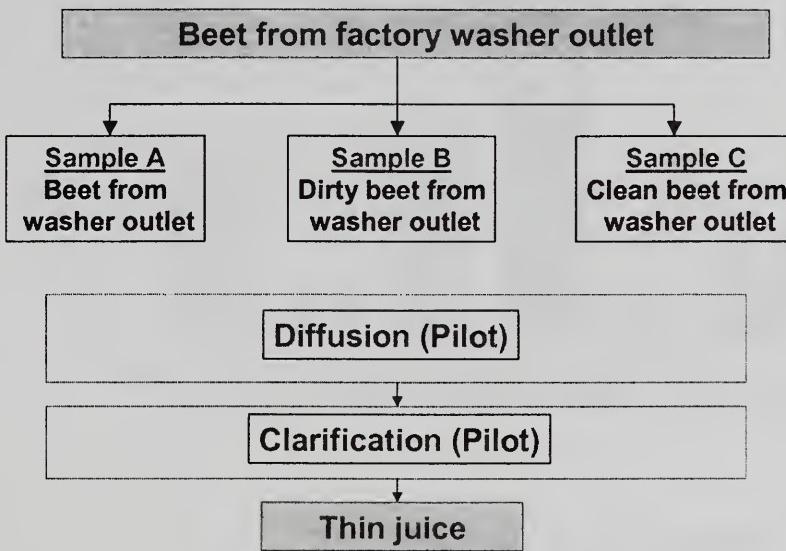
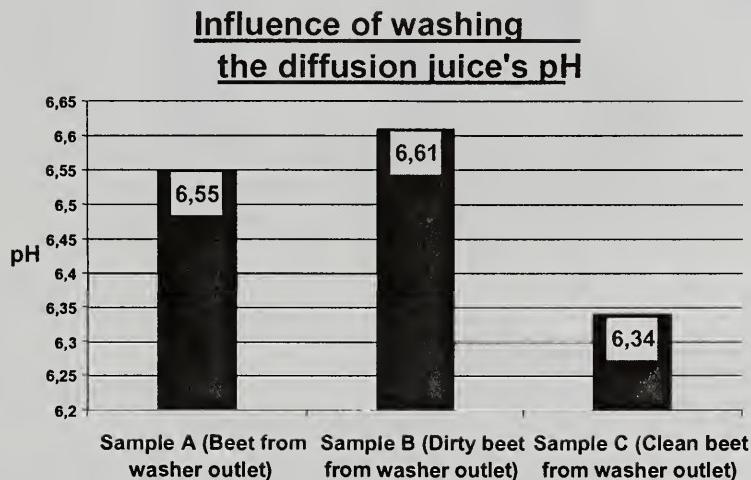


Figure 9. Time (field storage) vs. Sugar recovery (spring sown beets)

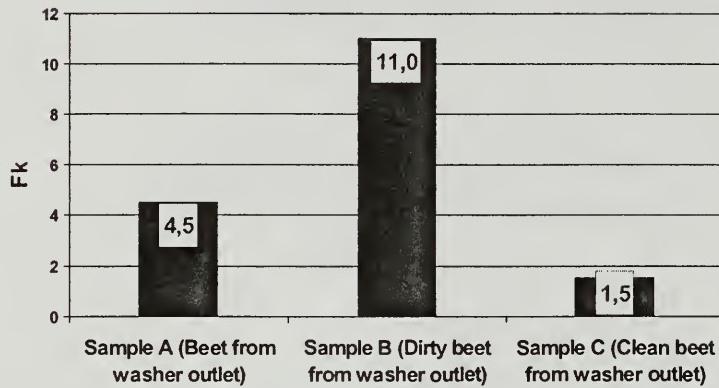
Beet washing efficiency. Pilot plant:



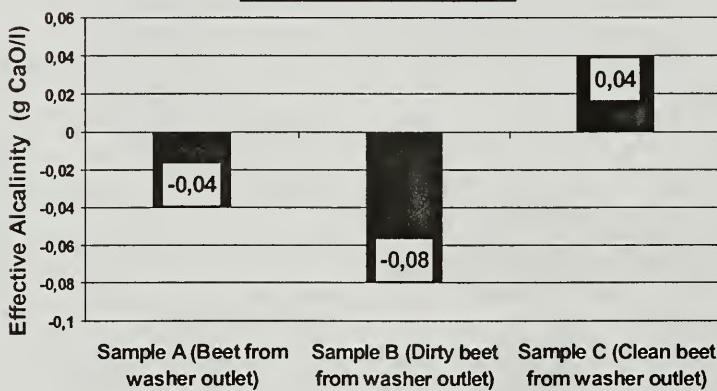
Beet washing efficiency. Results:



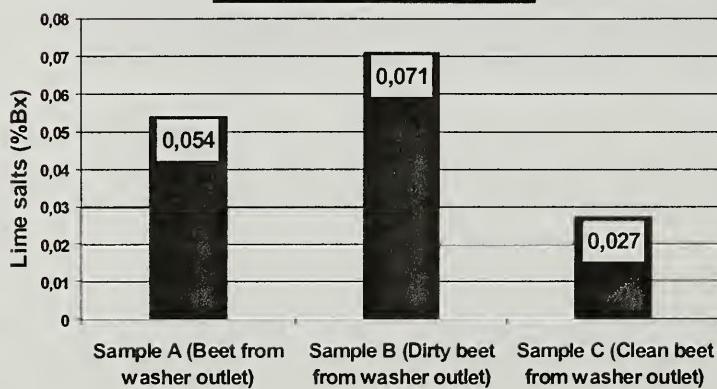
Influence of washing upon Juice Filterability

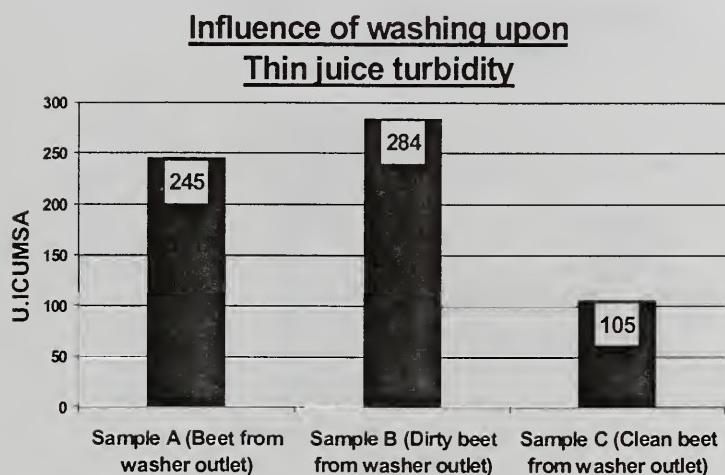
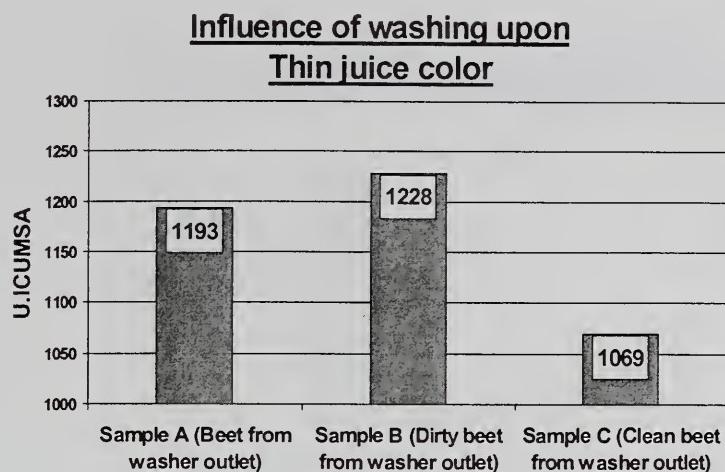


Influence of washing upon Effective Alkalinity



Influence of washing upon Lime salts in thin juice





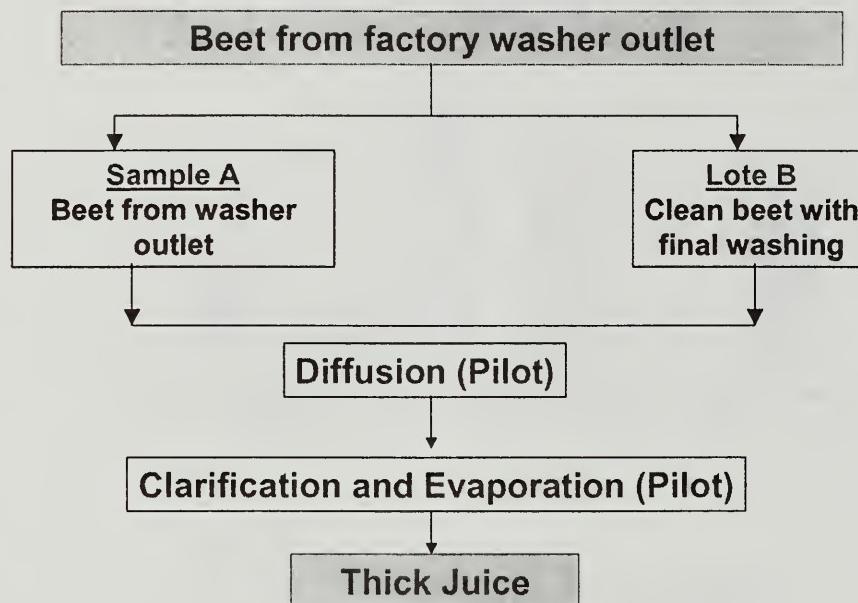
Beet washing efficiency. Summary:

The soil brought in by beets:

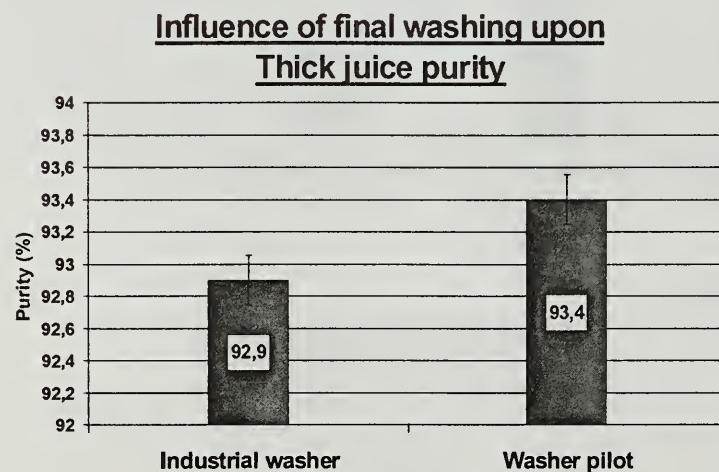
- Diffusion pH increases
- Filterability decreases
- Caustic soda consumption increases
- Thin juice color increases
- Thin juice turbidity increases

We should reconsider our well known washing drums, based on movement and a minimum of water. We need the contrary: more water present and time and less movement (friction among the beets)

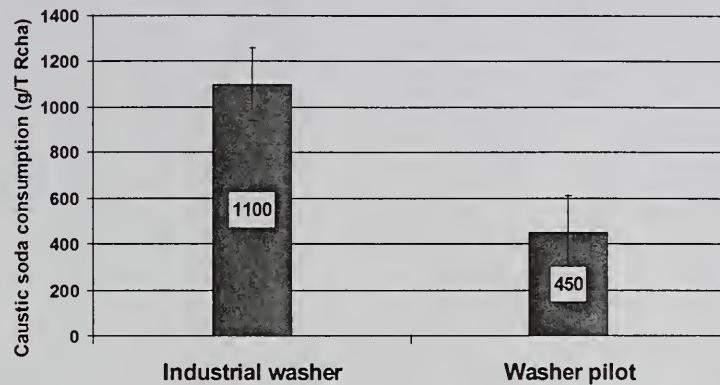
Final washing: wash water separation (spring sown beets). Pilot plant:



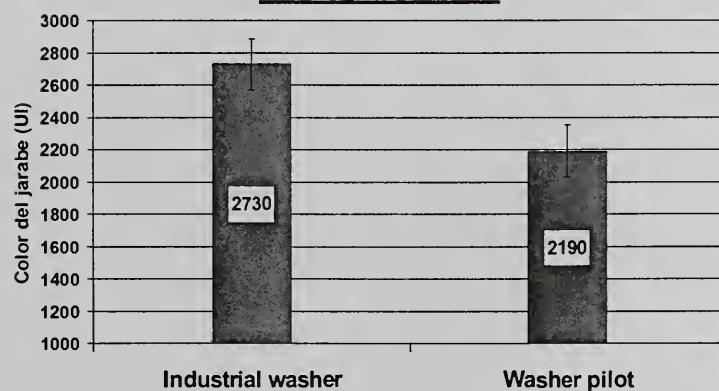
Final washing: Wash water separation (spring sown beets). Results:



Influence of final washing upon
Caustic soda consumption



Influence of final washing upon
Thick juice color



Furthermore:

- Sugar recovery increase: 1,4%
- Ash content decrease: 8%
- Thin juice turbidity decrease: 15%

CONCLUSIONS

- There are important business opportunities to be carried out.
- Not all the actions are complicated or cost intensive
- The industrial investments should be focused on the beets and their treatment
- This is one of the main parts of future competitiveness strategy

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